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VOLUME 39 SEPTEMBER 1961 NUMBER 5

SEXUALITY AND INTERFERTILITY STUDIES IN POLYPORUS ABIETINUS, P. PARGAMENUS, AND P. VERSATILIS[1]

B. K. BAKSHI AND T. G. CHOUDHURY

Abstract

The closely related fungi *Polyporus abietinus*, *P. pargamenus*, and *P. versatilis*, which were heterothallic and tetrapolar, were proved to be distinct species by interfertility tests. *P. versatilis* occurring on four species of timber showed multiple allelomorph heterothallism for both sex factors.

Polyporus abietinus Dicks. ex Fries and *Polyporus pargamenus* Fries exhibit similarities in their sporophores in the violet color of the hymenial surface when young and the presence of capitate cystidia. In both, the upper surface is white (becoming darker in *P. abietinus* when old), tomentose, and zoned. However, they are distinguished by habitat, *P. abietinus* usually occurring on conifers, *P. pargamenus* on hardwoods. The sporophores of *P. abietinus* are small, mostly effuso-reflexed and cracked, while those of *P. pargamenus* are large and sessile. Further, the tomentum on the pileus is strigose in the former and velvety in the latter. In culture, there is no definite basis for separating the two species, as also observed by Nobles (1948).

Polyporus versatilis (Berk.) Rom. is close to the two species discussed above, in the violet color of the hymenial surface and the presence of capitate cystidia. It is distinguished by its hispid surface, larger spores and pores. The spores and pores of *P. abietinus*, *P. pargamenus*, and *P. versatilis* are respectively 5.3–7 × 1.8–2.3 μ and 3–4 per mm, 4.3–5.1 × 1.7–2.1 μ and 3–5 per mm, and 5–7 × 3.3–4.2 μ and 1–3 per mm. *P. versatilis* causes distinct, white pocket rot in wood, while the wood decayed by *P. abietinus* and *P. pargamenus* is soft and spongy with no distinct pockets.

Such a closely related group of species presents an interesting subject for interfertility studies with a view to establishing their identity. Furthermore, undetermined sporophores, particularly when they are atypical, as in *P. versatilis* on kail in this study, or from unknown hosts, as in the case of *P. abietinus* and *P. pargamenus*, may be assigned to one species by interfertility tests. These studies including the occurrence of multiple allelomorph heterothallism in *P. versatilis* are reported in this paper.

[1]Manuscript received September 9, 1960.
Contribution from the Forest Pathology Branch, Forest Research Institute, Dehra Dun, India.

[The previous number of Can. J. Botany (**39**, 731–996) was issued August 2, 1961.]

Materials and Methods

In India, *P. abietinus* and *P. pargamenus* occur on dead wood of conifers and hardwoods, respectively, in the temperate region of the Himalayas. *P. abietinus* was collected on spruce (*Picea smithiana*) and *P. pargamenus* on oaks (*Quercus* sp.). *P. versatilis* occurs on both hardwoods and conifers in the tropics of the plains of India. The fungus was collected on dead wood of toon (*Cedrella toona*), *khair* (*Acacia catechu*), sal (*Shorea robusta*), and kail (*Pinus excelsa*). *P. versatilis* on kail was growing as small, resupinate sporophores on shingles treated with creosote and diesel oil and used as roofing on the Forest Research Institute building at Dehra Dun. The fungus, which did not form any pileus, typical of *P. versatilis*, was previously identified from sporophore (Bakshi, 1957).

The monospore cultures of the three fungi were obtained by the dilution plate method, in which the spore suspension, in increasing dilutions in sterile water, was poured on agar plates. After standing for about one hour, the water was drained off. Some spores were left behind on the agar plate. After incubation, the single germinated spores were spotted, one in each field, under the low-power of a microscope, cut off by a dummy objective cutter, and removed to malt agar slants in tubes.

The polyspore cultures of the three fungi possess clamp connections, which are absent in monospore cultures. The sexuality of each of the three species and also isolates from different hosts in *P. versatilis* was first determined by pairing monospore cultures from one sporophore in all possible combinations. The formation of clamp connections in paired mycelia indicated compatible matings, while their absence proved that the matings were incompatible. The symbol *AaBb* was used in representing the sexual composition of the compatible mycelia, which is heterozygous for both factors *A* and *B*. Interfertility tests among the three species were carried out by mating in all combinations selected monospore cultures belonging to four sexual groups within each species. The identity of *P. versatilis* on kail and the presence of multiple allelomorph heterothallism in *P. versatilis* were determined. The results, originally recorded in tables, are presented in abbreviated form.

Results of Tests

A. Sexuality Studies

1. Polyporus abietinus

AB: 1, 3, 4, 7, 10;	*aB:* not represented;
Ab: 8, 12;	*ab:* 2, 6, 9, 11, 13.

In listing homothallic and heterothallic Basidiomycetes (Whitehouse 1949) reported by various workers, *P. abietinus* is stated to be heterothallic and tetrapolar. This is apparently so in the present study, though one of the four sex groups is not represented.

2. Polyporus pargamenus

AB: 3, 4;	*aB:* 5, 6;
Ab: 1, 7, 8, 10, 11;	*ab:* 2, 9, 12.

P. pargamenus is heterothallic and tetrapolar as also shown by Nobles Macrae, and Tomlin (1957).

3. Polyporus versatilis

(*a*) Monospore cultures isolated from a sporophore on toon (*Cedrella toona*)

AB: 1, 9;	*aB:* 4, 8, 12;
Ab: 2, 5, 7, 11, 13;	*ab:* 6, 10.

(*b*) Monospore cultures isolated from a sporophore on *khair* (*Acacia catechu*).

AB: 2, 6, 10;	*aB:* 4, 8, 9, 11;
Ab: 3, 5, 7;	*ab:* 1, 12.

(*c*) Monospore cultures isolated from a sporophore on sal (*Shorea robusta*).

AB: 1, 2, 6, 8;	*aB:* not represented;
Ab: 7;	*ab:* 3, 4, 5.

(*d*) Monospore cultures isolated from a sporophore on kail (*Pinus excelsa*).

AB: 1, 4, 5, 6, 7;	*aB:* not represented;
Ab: not represented;	*ab:* 2, 3, 8.

P. versatilis is heterothallic and tetrapolar, although in isolates from sal, one sexual group is not represented. The sporophore on kail conforms to a bipolar type of sexuality. Interfertility tests with *P. versatilis*, however, proved that kail fungus is *P. versatilis* (two sexual groups are missing) so that it is really tetrapolar.

B. Identity of the Fungus on Kail

To establish the identity of the fungus on kail, the monospore mycelia of this fungus representing different sex groups were paired with those of *P. versatilis*, *P. abietinus*, and *P. pargamenus*. Clamp connections were formed only in pairings with *P. versatilis* and not with *P. abietinus* and *P. pargamenus*, proving the kail fungus to be *P. versatilis*.

C. Identity of P. abietinus, P. pargamenus, and P. versatilis

The monospore mycelia representing different sex groups of *P. abietinus*, *P. pargamenus*, and *P. versatilis* were paired in between these three species. For *P. versatilis*, monospore isolates from the four hosts were included. Clamp connections did not develop in any of the pairings, thus proving that the three species are distinct.

D. Multiple Allelomorph Heterothallism in P. versatilis

Monospore mycelia representing different sex groups of *P. versatilis* occurring on the four species of timber, namely: toon, *khair*, sal, and kail, were paired in all possible combinations. Clamp connections were formed in every pairing. This indicates the presence of multiple allelomorphs for both sex factors. The presence of identical allelomorphs for the single factor or both types of factor reducing the number of compatible matings was not demonstrated.

References

1. Bakshi, B. K. 1957. Decay in wooden shingles on building roof. J. Timber Dryers' and Preservers' Assoc. India, **3**, 24–25.
2. Nobles, M. K. 1948. Studies in forest pathology. VI. Identification of cultures of wood-rotting fungi. Can. J. Research, C, **26**, 281–431.
3. Nobles, M. K., Macrae, R., and Tomlin, B. P. 1957. Result of interfertility tests on some species of Hymenomycetes. Can. J. Botany, **35**, 377–387.
4. Whitehouse, H. L. K. 1957. Multiple-allelomorph heterothallism in the fungi. New Phytologist, **48**, 212–244.

CONTRIBUTION TO THE EMBRYOLOGY OF HOPPEA DICHOTOMA WILLD. (GENTIANACEAE)[1]

GOVINDAPPA D. AREKAL

Abstract

The mode of embryo sac development in *Hoppea dichotoma* Willd. conforms to the Polygonum type. No integumentary tapetum is organized around the female gametophyte. The organization of the endosperm follows the nuclear type. Simultaneous cell wall formation occurs throughout the endosperm when the embryo is at the 4- to 6-celled stage. The development of the embryo is assigned to the second period, V megarchetype and group 11 in series C′ in the system of embryogenic classification of Souèges. The endosperm and embryo of Gentianaceae are compared with those of Menyanthaceae.

Introduction

The results of embryological investigations in the Gentianaceae so far have demonstrated that the aquatic genera *Menyanthes* and others treated as a tribe, Menyantheae, in the Gentianaceae by Bentham and Hooker (1) and as a subfamily, Menyanthoideae, by Gilg (8) merit the rank of a separate family Menyanthaceae as classified by Don (7), Wettstein (20), and Hutchinson (10).

Schnarf (14) considered Gentianaceae and Menyanthaceae as independent families and gave a summary of the early embryological work on them in his "Vergleichende Embryologie der Angiospermen". The later investigations include that of Stover (19) on the life history of *Nymphoides peltatum*, Srinivasan (15) on the cytomorphological study of *Limnanthemum cristatum* and *Enicostemma littorale*, Souèges (17) on the embryogeny of *Menyanthes trifoliata*, McCoy (12) on the embryology of *Swertia carolinensis*, and Crété (2, 3, 4, 5, 6) on the development of the embryo in *Gentiana asclepidea*, *Erythraea centaurium*, *Chlora perfoliata*, *Limnanthemum nymphoides*, and *Gentiana punctata*. The present work, which deals mainly with the formation of the endosperm and embryo in *Hoppea dichotoma*, was undertaken because there is no embryological information on the genus *Hoppea*.

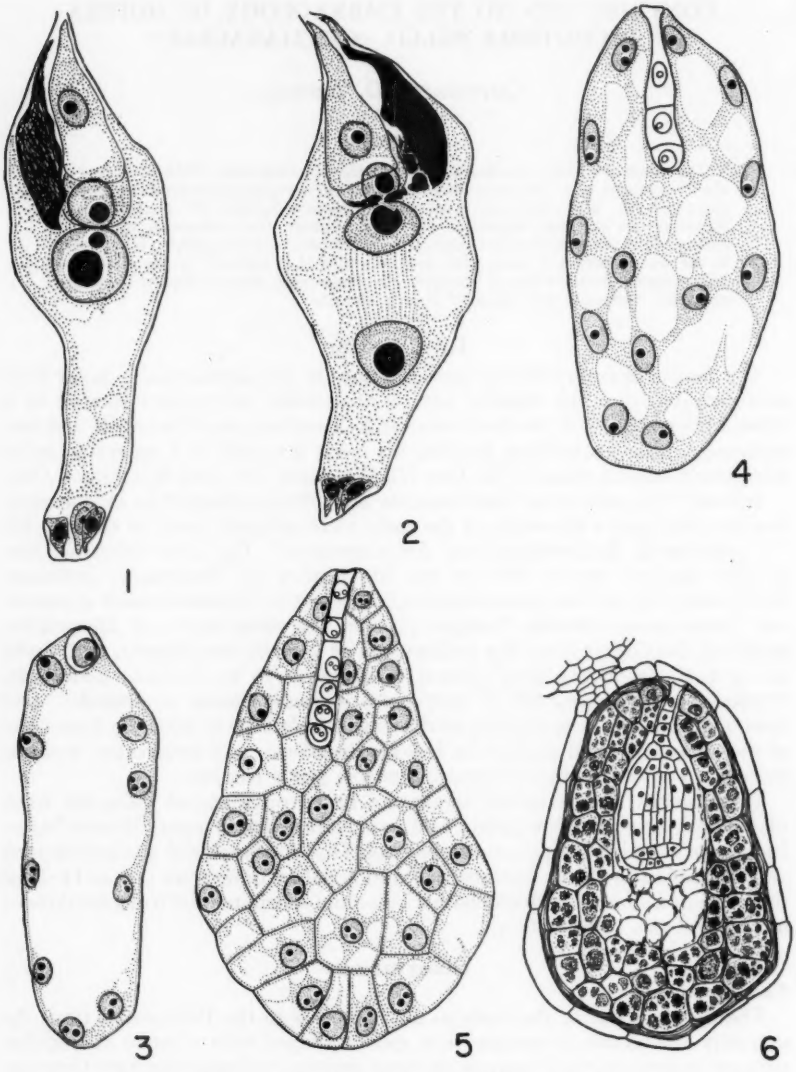
The observations recorded herein are based on material collected from plants growing on a fallow paddy field near Narasimharajapura, Mysore State, India, and fixed in formalin–acetic–alcohol. The killed material was dehydrated and embedded in the customary way and serial sections were cut at 12–14 μ thickness and stained in Heidenhain's iron-alum-haematoxylin using erythrosin in clove oil as a counterstain.

Observations

Endosperm

The development of the embryo sac conforms to the Polygonum type. At maturity the female gametophyte is spindle-shaped with a broad micropylar part and a comparatively narrow chalazal portion. An integumentary tapetum

[1]Manuscript received April 23, 1961.
Contribution from the Department of Botany, University of Toronto, Toronto, Ontario.



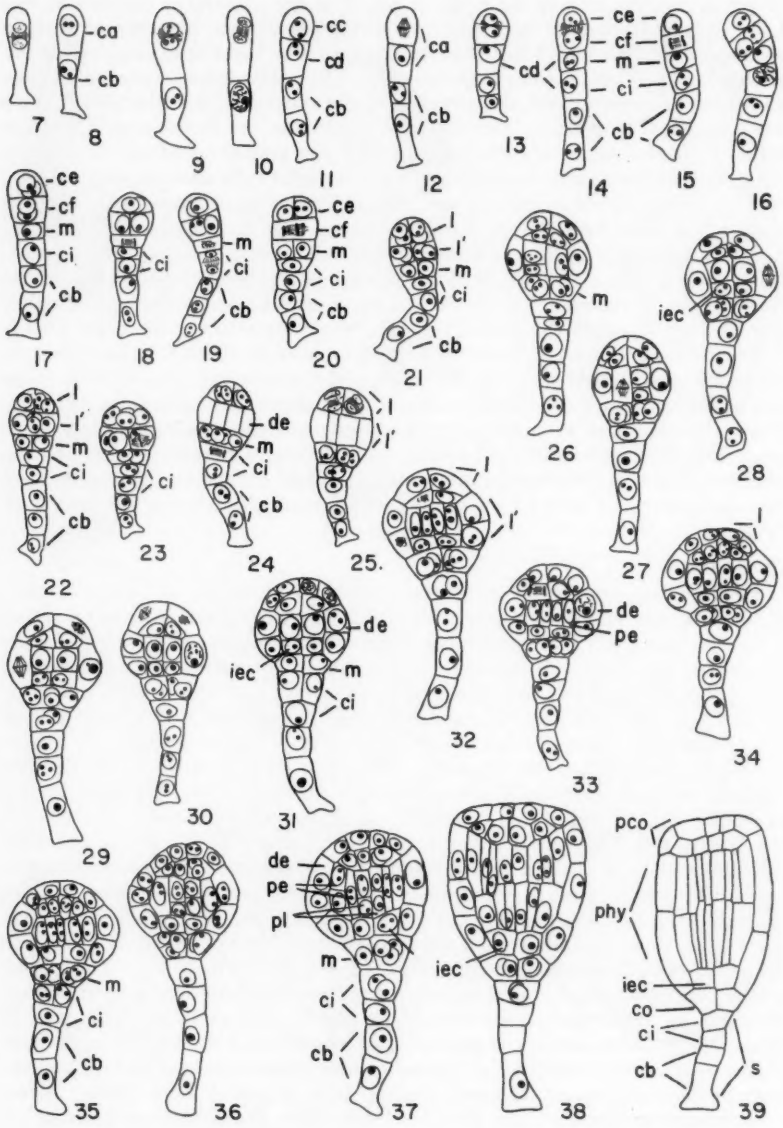
FIGS. 1–6. Fig. 1. A stage in double fertilization, ×900. Fig. 2. First division of the primary endosperm nucleus, ×900. Fig. 3. L.s. embryo sac with young endosperm; note the central vacuole, ×630. Fig. 4. L.s. embryo sac with free nuclear endosperm after the reorganization of the central vacuole, ×630. Fig. 5. L.s. embryo sac with endosperm after cell wall formation, ×630. Fig. 6. L.s. mature seed, ×450.

is not organized around the embryo sac. The egg apparatus consists of two large synergids and an egg. The two polar nuclei fuse together to form a secondary nucleus which lies close to the egg. The three antipodal cells have pointed ends directed towards the chalaza. The pollen tube enters the embryo sac at the micropylar end, destroys one of the synergids, and liberates the two male nuclei. One of them fuses with the egg giving rise to the zygote and the other with the secondary nucleus forming the primary endosperm nucleus (Fig. 1). The surviving synergid and the antipodal cells soon degenerate.

The primary endosperm nucleus divides earlier than the zygote. No cell wall is laid down between the daughter nuclei (Fig. 2). The two nuclei move apart and undergo a series of simultaneous free nuclear divisions to produce a large number of nuclei which lie in the thin peripheral layer of cytoplasm around a large central vacuole (Fig. 3). As the number of nuclei increases by further free nuclear divisions the central vacuole breaks up into smaller ones. This is followed by an increase in density of the cytoplasm in which the nuclei remain uniformly distributed (Fig. 4). When the embryo is at the 4- to 6-celled stage cell walls are laid down simultaneously throughout the endosperm (Fig. 5). During later stages of endosperm development these cells accumulate large quantities of deep-staining substances and the outer tangential walls of the peripheral cells acquire prominent lamellated thickenings. In the mature seed the endosperm is covered by a single layer of cells representing the seed coat (Fig. 6).

Embryo

The fertilized egg divides transversely after the formation of a large number of nuclei in the endosperm and gives rise to the two-celled proembryo (Figs. 7, 8). The terminal cell *ca* undergoes a transverse division resulting in the cells *cc* and *cd*. The basal cell *cb* after a similar division produces two superposed cells (Figs. 9–11). The primary tetrad consists of a row of four cells and consequently conforms to the C_2 category (16). The cells *cc* and *cd* also divide transversely and give rise to cells *cf* and *ce* and *ci* and *m* respectively (Figs. 12–14). A vertical division occurs in cell *cf* and then in *ce* (Figs. 15–19). By another vertical division in the same sequence but at right angles to the previous one two tiers of four cells each are organized at the terminal region of the proembryo. They are designated as *l* and *l'* (Figs. 20–22). Periclinal divisions take place in the tier *l'* and delimit the dermatogen, *de*, from a group of inner cells which soon divide transversely giving rise to two tiers of cells (Figs. 23–31). While the cells of the basal tier constitute the initials of the root cortex, *iec*, those of the terminal tier undergo periclinal divisions and produce the periblem, *pe*, and plerome, *pl* (Figs. 32–37). The cells of tier *l* contribute to the stem tip and cotyledons, *pco*, by undergoing periclinal and anticlinal divisions as the hypocotyledonary part *phy* is organized from the tier *l'* (Figs. 25–39). Meanwhile cell *m* undergoes two vertical divisions one at right angles to the other resulting in a group of four circumaxial cells which contribute to the root cap, *co*. The cells derived from *ci* and *cb* form a short, often uniseriate suspensor, *s* (Fig. 39). The embryo in the mature seed remains feebly developed with rudimentary cotyledons.



FIGS. 7–39. These show the stages in the development of the embryo, ×630.

Discussion

The nuclear type of endosperm development observed in *Hoppea dichotoma* has been recorded in all the species of Gentianaceae so far investigated except two of the saprophytic forms, viz., *Voyria coerulea* and *Voyriella parviflora*, in which it is cellular (13). Wall formation takes place in the present species after a large number of free nuclei are formed and the proembryo consists of a few cells as in *Gentiana campestris* (18) and *Swertia carolinensis* (12). However, according to Oehler (13) it occurs at the eight-nucleate stage in *Leiphaimos spectabilis* and eight- or more-nucleate stage in *Cotylanthera tenuis* before the first division of zygote. Nevertheless, the simultaneous type of wall formation observed throughout the endosperm in the present study is similar to that of *Leiphaimos spectabilis* and *Cotylanthera tenuis* (13). It is unlike *Gentiana campestris* (18) and *Swertia carolinensis* (12) in which cell walls appear first at the peripheral part and then extend to the center of the embryo sac.

While a large majority of Gentianaceae thus far studied have a nuclear type of endosperm development the Menyanthaceae possess only the *ab initio* cellular type (12, 13, 15, 18).

The apical cell *ca* of the two-celled proembryo of *Hoppea dichotoma* is wholly responsible for the production of the embryo proper and the basal cell *cb* furnishes only a few cells which go to form a part of the suspensor. A similar behavior of the cell *ca* has been recorded in *Gentiana asclepiadea*, *G. punctata*, *Erythraea centaurium*, and *Chlora perfoliata* (2, 3, 4, 6). The cell *m* in the present species produces a tier of four circumaxial cells representing the root cap as in *Erythraea centaurium* and *Chlora perfoliata* (3, 4). But in *Gentiana asclepiadea* and *G. punctata* (2, 6) it gives rise to two tiers of four cells each which again divide several times organizing a well-differentiated root cap. Furthermore, cells *ci* and *cb* also undergo a considerable number of divisions to produce a massive, very often quadriseriate, suspensor as against a delicate and invariably uniseriate suspensor of *Erythraea centaurium*, *Chlora perfoliata* (3, 4), and *Hoppea dichotoma*.

In *Hoppea dichotoma*, like *Gentiana asclepiadea*, *G. punctata*, *Erythraea centaurium*, and *Chlora perfoliata* (2, 3, 4, 6), the first proembryonal tetrad is linear, conforming to the C_2 category. Moreover the development of the embryo belongs to the second period, V megarchetype and group 11 in series C′ in the system of embryogenic classification of Souèges (16). On the other hand, in *Menyanthes trifoliata* (17) and *Limnanthemum nymphoides* (5) of Menyanthaceae, the first tetrad is T-shaped and of the A_2 category and because the cell *ca* of the two-celled proembryo contributes only to the stem tip and cotyledons, and the rest of the embryo is derived from the basal cell *cb*, the development belongs to the first period, II megarchetype and group 1 in series A. The laws governing the embryogeny of Gentianaceae are therefore entirely different from those of Menyanthaceae. The differences between Gentianaceae and Menyanthaceae are not limited to such features as the development of endosperm and embryo but extend as well to the nature of the pollen sac (9), floral anatomy (11), form of vascular bundle, and also to external features such as the position and appearance of leaves.

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References

1. Bentham, G. and Hooker, J. D. 1876. Genera Plantarum. Vol. 2. London.
2. Crété, P. 1949*a*. Embryogénie des Gentianacées. Développement de l'embryon chez le *Gentiana asclepiadea* L. Compt. rend. **228**, 768–770.
3. Crété, P. 1949*b*. Embryogénie des Gentianacées. Développement de l'embryon chez l'*Erythraea Centaurium* Pers. Compt. rend. **228**, 1448–1449.
4. Crété, P. 1955. Embryogénie des Gentianacées. Développement de l'embryon chez le *Chlora perfoliata* L. Compt. rend. **241**, 1825–1828.
5. Crété, P. 1956. Menyanthacées. Développement de l'embryon chez le *Limnanthemum nymphoides* Hoffg. et Link. Compt. rend. **242**, 3110–3113.
6. Crété, P. 1959. Embryogénie des Gentianacées. Développement de l'embryon chez le *Gentiana punctata* L. Compt. rend. **248**, 3594–3596.
7. Don, C. 1938. General history of dichlamydeous plants. Vol. 4. London.
8. Gilg, E. 1895. Gentianaceae. *In* Die natürlichen Pflanzenfamilien. *Edited by* A. Engler and K. Prantl. Vol. 4. Leipzig.
9. Guérin, P. 1926. Le développement de l'anthere chez les Gentianacées. Bull. soc. botan. France, **73**, 5–18.
10. Hutchinson, J. 1959. Families of flowering plants. Vol. 1. Dicotyledons. Oxford.
11. Lindsey, A. A. 1938. Anatomical evidence for the Menyanthaceae. Am. J. Botany, **25**, 480–485.
12. McCoy, R. W. 1949. On the embryology of *Swertia carolinensis*. Bull. Torrey Botan. Club, **76**, 430–439.
13. Oehler, E. 1927. Entwicklungeschichtlicht-zytologische Untersuchungen an einigen saprophytischen Gentianaceen. Planta, **3**, 641–733.
14. Schnarf, K. 1931. Vergleichende Embryologie der Angiospermen. Berlin.
15. Srinivasan, A. R. 1941. Cyto-morphological features of *Limnanthemum cristatum* Griseb. and *Enicostemma littorale* Blume. Proc. Indian Acad. Sci., B, **14**, 529–542.
16. Souèges, R. 1939. Embryogénie et classification. Hermann et Cie, Paris.
17. Souèges, R. 1943. Embryogénie des Gentianacées. Développement de l'embryon chez le *Menyanthes trifoliata* L. Compt. rend. **217**, 488–490.
18. Stolt, K. A. H. 1921. Zur embryologie der Gentianaceen und Menyanthaceen. Kgl. Svenska Vetenskapsakad. Handl. **61**, 1–56.
19. Stover, E. L. 1932. Life history of *Nymphoides peltatum* (S. P. Gmel) Britten & Rendle. Botan. Gaz. **93**, 474–483.
20. Wettstein, R. 1935. Handbuch der systematischen Botanik. Vol. 2. Leipzig.

CRITIQUE OF SOME VEGETATIONAL CONCEPTS AS APPLIED TO FORESTS OF NORTHWESTERN ALBERTA[1]

J. S. ROWE

Abstract

The application, by R. Plochmann, of certain European silvicultural and ecological concepts in the study and description of forests of northwestern Alberta is examined. His ideas of unidirectional succession to the single climax forest are criticized on the grounds that concepts such as *succession* and *climax* must always be related to specific terrain. Every forest community, and indeed every vegetational community, is the botanical part of a unique geographic ecosystem which gives it meaning, and it is neglect of the geographic framework which has led to the false assumptions that forest communities can be studied as things-in-themselves, and that vegetational concepts derived in one region can be transplanted unchanged to others.

Introduction

It is always interesting to see a familiar scene through the eyes of a stranger, to get the first impressions of someone with a different background and hence with a different perspective. Dr. Richard Plochmann, a German-trained forester, has written an ecological account of forests in northwestern Alberta, and there are novel and stimulating aspects both to his observations and to the viewpoint from which they have been made. In coming to Canada, Plochmann's purpose was to study some of the natural forests of the north. He was able to gather data for his report in the Notikewin–Keg River area in 1953, while employed by the Forest Survey Division of the Alberta Department of Lands and Forests. Hitherto there have been few reports on forest development in this general area (see Moss 1953, 1955).

The article "Stand structure and tree succession in northern virgin forests"[2] calls for commentary on two counts. First, the underlying concepts of the work are of particular interest, for they derive from a contemporary school of European thought whose influence is felt not only in silviculture but also in the broader fields of vegetation science (phytosociology) and soil science. Second, as an assessment of forest conditions in the mid-western boreal forest region, the information concerning silvics of species, site influences, and conditions of stand development needs to be examined and evaluated.

In the "Journal of Ecology" Jones (1959) has briefly reviewed the article, pointing out some of its controversial aspects. His closing sentences provide a good introduction to the present paper: " . . . the picture which Plochmann presents is based on visual impressions and must be to some extent subjective. It is plausible and convincing and is in accordance with much current European thought. Plochmann has made very good use of his

[1]Manuscript received December 15, 1960.
Contribution from Canada Department of Forestry, Forest Research Branch, Ottawa, Ontario.

[2]"Bestockungsaufbau und Baumartenwandel nordischer Urwälder". Richard Plochmann, 1956 (German text, summary in English). Forstwiss. Forsch. Hefte **6**, 1–96. (Beih. zum Forstwiss. Centr.)

opportunities and has evidently been an acute and intelligent observer . . . But he might have seen mainly what he looked for, and only some of those who know the forests intimately can say how true the picture is. It is to be hoped that Plochmann's excellent and fascinating account will stimulate Canadian foresters either to corroborate or to disprove his hypotheses."

Theoretical Background

In order to understand Plochmann's approach to the study of the Alberta forests, it is necessary to present a background of ideas that are today espoused by an important group of European silviculturists. One recurrent theme is that of the virgin or natural forest as the basis and source of rules for a rational forest management (for example, see Vezina 1959). Such forests are conceived as attuned to Nature, existing in healthy and harmonious equilibrium with climate and with soil. In them man finds the embodiment of the "laws of existence" and in the words of Leibundgut (1959): "Forest science is interested, first of all, in forests that have remained untouched by human influences and which, therefore, manifest most clearly the natural regularities of their structure and dynamics. For this reason virgin-forest research has become the principal concern of fundamental research regarding silviculture and yield".

The idealization of the natural state has its roots in disillusionment with man's abilities to manage the earth's resources well. Köstler (1956), for example, says: "Centuries ago the forest originated on the soil without man's assistance; it grew in the still unweakened productive power of the earth and, undisturbed by the senseless and rapacious onslaughts of mankind reached a degree of perfection which nowadays must be regarded only as exceptional, and on the whole no longer attainable." And again, quoting Rousseau: "All is well when it leaves the hands of the author of all things, everything degenerates in the hands of man".

The accusation is just. Man stands convicted of careless mismanagement or outright plunder of the earth, as many books on conservation attest. Yet, granting past sins, one may still be skeptical of the idealization of the natural state (Peace 1960). In the forest, as elsewhere, virginity is not always accompanied by excessive virtue. The harmony-of-nature concept romanticizes a status quo in which man plays no part, and doubtless it holds more charm for those who lack untouched vegetation than for those of us who can go out and see (frequently with dismay) what theory assures us ought to be a beautifully balanced condition: healthy, harmonious, and in every way ideal. It is notable that Köstler (1959) reported, after a recent visit to the Gaspé region in Quebec, that snow, wind, and insects take their toll of the virgin balsam fir forests despite their unquestioned "naturalness".

The natural forest is further conceived as the end point of all successional series, the climax "organism" to which the various younger forest communities inevitably mature. This point of view is close to the monoclimax theory of F. E. Clements, and it has been vigorously challenged, particularly in the last decade in North America (see Whittaker 1953). One of the strongest counterarguments points out that relationships within any community are social (i.e. mediated through environment) rather than organic, and

therefore there is no successional certainty comparable with the development of the individual organism. A large element of chance enters into forest succession, and the changes that occur with the passage of time in the vegetation pattern of an area are governed by the vagaries of history, by local and surrounding site conditions, and by ecological tolerances of the species that happen to be available.

Finally, there is emphasis on the spatial organization or physical "structure" of forest stands. European forests are intensively managed, and attention is focused on the individual stand or even on the individual trees. As sustension is a primary consideration, silvicultural treatment is oriented to the manipulation of stand structure. The aim of the prescribed cut is to harvest some fraction of the standing wood while therapeutically improving that which remains. In European forestry journals this commendable concern with structure is reflected in the frequent use of stand profile and crown projection sketches, and in classification of individual tree forms and community "phases".

The concepts of structure are tied in neatly with the organismic concept of the natural or climax forest. The latter is conceived as in dynamic equilibrium; it maintains a steady state of continual cyclic change whereby, patchwise within the forest, the phases of maturity pass to those of old age, then to youth, and back to maturity once more. Each vital phase of the cycle can be recognized by a particular structure form, as can the earlier stages of succession before the climax is reached. It is the purpose of forest research to discover the developmental processes and the life rhythms of the natural forest, as expressed by structural stages and phases with their "genetic" relationships. Quoting Lamprecht (1954): "Only when the natural stand structure and its developmental tendencies are known, can one reliably formulate the appropriate management goals and work toward their realization through concrete rules . . ."

Climax Forest in Northern Alberta

With this introduction, Plochmann's ideas will now be outlined. He sees the climax forest type of the upland (mineral) soils in northern Alberta as *white spruce – balsam poplar – fir* (*Picea glauca – Populus balsamifera – Abies balsamea*), strongly dominated by the spruce and with constant though relatively small quantities of the balsam poplar and fir. Also present in limited amounts are white birch (*Betula papyrifera*) and trembling aspen (*Populus tremuloides*), and infrequently the jack pine (*Pinus banksiana*) and lodgepole pine (*Pinus contorta* var. *latifolia*). The climax is believed to be uniform despite variations in relief, exposure, and other site features, and this is attributed to the equalizing effect of the soil which is presumed to develop to a homogeneous state (pedoclimax) under the influence of the stable environmental conditions.

The spruce–poplar–fir type is not static. It exists in a state of dynamic change, constantly developing, aging, renewing. At any one locality the cycle is completed in about 200 years, during which period three alternating structural phases can be distinguished: the optimal phase, the aging phase, and the young phase. The optimal phase is the most durable of the three, extending

over a period roughly the equivalent of the young and old phases together. It is recognized as a many-layered, irregular-canopied forest with an overstory of tall white spruce, interrupted here and there by single stems or groups of balsam poplar and aspen, a middle layer consisting mostly of spruce but with sparse fir and birch, and an extremely mixed understory which features, along the gradient of decreasing shade, fir, spruce, birch, balsam poplar, and aspen. Also related to the gradient of light is the composition of the minor vegetation, with moss cover in the deep shade and with tall shrubs and herbs in the openings.

The forest is uneven-aged, and frequency curves of stem diameters (all species together) show the typical reversed-J form. Both the density and distribution of the growing stock is at the optimum, with 18%, 53%, and 29% of the volume (5360 cu. ft per acre) in the diameter classes 4–12 in., 13–20 in., and >20 in., respectively. Approximate values for basal area and annual volume increment are 150 sq. ft and 80 cu. ft per acre. These numerical characteristics, says Plochmann, are astonishingly similar to samples from natural and managed forests in the mountains of middle and southern Europe.

The aging phase commences, when, owing to shading effects of the spruce canopy and the accumulation of "raw" needle humus, the regeneration of the various species fails and the mixed understory of young trees disappears. Then takes place a gradual transition to a more uniform, single-layered structure in which spruce is more prominent than ever, although total forest productivity is reduced by about 20%. Despite the trend toward structural uniformity, the uneven-aged character is maintained. Finally, as trees fall out from old age or wind, a gappy structure characterizes the decadent forest.

Plochmann theorizes that with the appearance of openings in the forest pattern, the young phase is initiated. Grasses and shrubs invade the gaps and "consume" the raw humus, allowing entrance of the frost-hardy leaf trees, the birch, aspen, and balsam poplar. These pioneers ameliorate the local climate by reducing the incidence of frost, and in the shelter of the nurse crop, the spruce springs up. It soon pushes through the broad-leaved canopy and asserts its former dominance. The extended period of regeneration from the aging to the young phase, the carry-over of old trees from the former phase, and the marked tendency of the spruce to differentiate in age, all serve to reinstate an uneven structure with resemblances to the group selection ("Femel") forest. Because of the marked concentration of stems in the lowest class, there is small depreciation and a high annual growth increment which exceeds even that of the next, the optimal phase. In time, the size distribution of stems gradually shifts to the middle and upper diameter classes, the storeyed structure is rebuilt, and the optimal phase is again reached to complete the life cycle of the natural forest. The three-phase cycle goes on continuously, slowly or rapidly on small areas, conferring on the climax spruce–poplar–fir type three outstanding characteristics: stability of composition, constancy of development, and high production capacity.

This picture of the climax boreal forest fits the European concept of the virgin forest perfectly, and we are justified in suspecting that the data may unconsciously have been adapted to preconceived notions. The "genetic" relationship of "structural phases" in a "life rhythm of the community"

is a faith difficult to substantiate. It is of course possible to group sample plots in such a way as to give credence to it, and apparently Plochmann has not recognized the circularity inherent in such an approach. Inconsistencies, such as the wide variability of plots assigned to the same phase, or the unexpected increase in balsam poplar in the decadent (aging) phase, he attributes to imperfect sampling. It needs to be pointed out, moreover, that averaged enumerations of plots tell little about actual values or spatial relationships; for example, irregular diameter distributions on the tally sheet may or may not reflect irregularities of stand structure (Jones 1945). In short, mathematical analysis and synthesis of tree and plot measurements can be used neither to prove nor to disprove the hypotheses set forth.

Against Plochmann's thesis, let me propose another based on first-hand familiarity with some parts of the boreal forests in Manitoba, Saskatchewan, Alberta, and the Northwest Territories: There is no single climax forest community characteristic of upland mineral soils; rather, there are numerous types—some transitional, some relatively persistent—which are fitted ecologically to particular types of land. Short-lived ecological types (forest-land types) are characterized by the intolerant species, pine, aspen, balsam poplar, and birch, while the persistent ecological types are so because their species are more tolerant and, in the case of spruce (though not the fir), longer lived. Only balsam fir has the ability to consistently reproduce itself on the forest humus under a closed canopy of the longer-lived trees, but as this species is otherwise poorly adapted to the relatively dry continental environment its importance in the general forest picture is minor. By default the white spruce dominates many upland sites, although it is not strongly self-perpetuating. Like the pine and birch it shows pioneer tendencies in its invasion of grassland, cleared mineral soil, or burned-over lands, in its optimum growth where overhead shade is slight, and in the difficulty with which it regenerates in its own shade or in that of other species. In short, there are no species in the Canadian western boreal forest possessing in full the silvical characteristics appropriate to participation in a self-perpetuating "climax". The western boreal forest is a disturbance forest, usually maintained in youth and health by frequent fires to which all species, with the probable exception of fir, are nicely adapted. Decadent forests do not return through an inevitable cycle to youth and an optimum phase but rather tend to remain open, unhealthy, ragged, and frequently brush-filled, awaiting the rejuvenating touch of fire, flood, or windfall-ploughing of the soil.

For this thesis there is negative support as well as positive evidence. Thus during nine years of work in the western boreal forests I never saw the type of spruce stand which in physiognomy or condition of humus layer would suggest a third or fourth generation forest. Irregular-structured, humocky-floored stands which do fit the description can be observed in the more humid parts of the eastern Canadian boreal forest and they are distinctive.

The irregularity of structure in old forests, where not due to partial burning, results from the filling-in process whereby spruce groups or single trees occupy the gaps that open where the shorter-lived leaf trees, or pine, fall out. Such filling-in may be accomplished rapidly where initial disturbance and site conditions favor strong immediate invasion of spruce, or slowly and over a

long period where environment is favorable to establishment and longevity of aspen, poplar, birch, or pine. Once a spruce overstory is formed the stands tend to stagnate, and further changes consist mainly of an increase in the incidence of balsam fir plus a sporadic "dribbling in" of spruce on favorably located rotted stumps and logs as the old trees of the first generation die out.

At any rate, sequences of forest phases or stages have limited meaning apart from type of land; for example, the spruce forest that follows balsam poplar on alluvial flats should not be grouped with that which outlasts the pine, aspen, and birch on surface-dry siliceous till, or with that which follows aspen on fresh (mesic) calcareous till.

Forest Fires and Succession

Having cast a skeptical eye over Plochmann's boreal climax hypothesis, and having made the preliminary suggestion that in any considerations of vegetational change the associated type of land cannot be neglected, we turn next to the second part of his paper dealing with the successional stages which, following fire, are presumed to lead back to the spruce–poplar–fir climax.

Plochmann rightly recognizes fire as a normal feature of the boreal forest, one which intermittently interrupts the "harmonious, balanced rotation". For the individual stand it is a catastrophe; for the forest as a whole, it has its natural place. The most important effect of fire, he believes, is on the moisture regime of the surface soil: dry sites are made drier and wet sites wetter (frequently with swamping). Thus site differences obscured under the climax forest are sharpened following fires, and the only clearly favorable effect of the latter is destruction of the raw humus that prevents regeneration.

The picture he presents of succession after fire shows a gradual replacement of the even-aged pioneer stands of pine or leaf trees (aspen, poplar, birch) by a mixed, storyed forest in which white spruce plays an increasingly important role, leading to the formation of an irregular, uneven-aged spruce stand, something like a single tree selection ("Plenter") forest, which culminates in the climax spruce–poplar–fir type. If fire does not again intervene, the direction of the process is inevitable, speeded through its early stages by the activity of bark-eating animals (rabbits, porcupine, moose) which open the pine and aspen stands to invasion by the spruce, and slowed or accelerated according to site conditions which determine the aggressiveness of the invader once there. In turn the spruce is responsible for progessively changing stand structure from a regular to an irregular form, and with each succeeding generation the growth capacity of stands increases.

On dry sites, such as ridges or upper slopes, Plochmann believes that the pioneer forests of pine or aspen are regularly dense and closed, and despite openings caused by snow breakage, by bark-scaling animals, and by fungus attack (especially *Hypoxylon pruinatum* and *Fomes igniarius* on the aspen), the invasion of spruce is slow owing to intense root competition for the limited supplies of moisture and nutrients. The climax may not be attained until the fourth or fifth generation of forest (300 to 500 years). On the other hand, on intermediate (mesic) sites the spruce rapidly invades the aspen–poplar fire stands, and a variable structured and patchy mixed forest is attained by the second forest generation. Similarly on wet sites, the spruce soon succeeds

the willow, alder, and deciduous trees, and progress to the climax takes place in a matter of three or four forest rotations (200 to 400 years).

In developing his interpretation of postfire succession, Plochmann has brought up ideas that merit further study: His singling out of bark-eating animals as the important agent in hastening the transition to spruce forest (although the role of one of the most important animals—the beaver—is not mentioned), and his assessment of snow breakage as a factor both in the opening of dense aspen stands and in the top-dying of balsam poplar. His thesis that it is the "frost pocket" condition of stand openings which deters spruce invasion, and the relating of regeneration of the various tree species to size-of-gap in parallel with their tolerances of shade. Also the statement that spruce makes its best growth in long-undisturbed stands.

At the same time, however, the argument is weakened by a number of questionable assumptions and misconceptions: for example, that aspen and balsam poplar regularly regenerate by seed on burned areas and in large forest openings, that the densest and most regular pioneer forests of pine and aspen form on dry sites where white spruce is said to invade with most difficulty, that undershrubs grow most vigorously under an aspen canopy and that the shrub layer weakens when the tree overstory disintegrates, that white spruce always requires a "nurse crop" of leaf trees to capture the ground for it, and that "raw humus" accumulates under spruce on gray-wooded soils. Apparently a homology with acid podzols is assumed here. Furthermore, there are a number of important factors not taken into consideration, such as the influence of fire severity in providing either mineral soil or scorched humus as the seed bed for spruce, the role of decayed wood on the forest floor as a favorable medium for survival of seedlings, the smothering effects of leaf-tree litter on young conifers, and the effects of rabbit browsing of spruce seedlings and saplings under brush and leaf-tree cover (Rowe 1955).

The composition and productivity of stands in the different stages or "generations" of succession are described, the data being derived in the same way as for the climax phases, namely by grouping and averaging sample plots that *a priori* are believed to stand in certain relationships to one another. The procedure is frankly subjective as Plochmann admits, and one can question as well the validity of the identification of second, third, and fourth "generations" of the forests by what is surely an indefinite criterion: the position of charcoal in the humus layer and upper soil.

The fundamental criticism must however be directed to the concept of succession as a purely vegetational process, running on unidirectionally to a predetermined end with only ephemeral influence by physiographic site. Succession is what happens with space fixed and time changing, and it can only have specific meaning in the context of a particular geographic framework. If it is known what species are present as well as what ones are in the vicinity of a particular land area, and if something of the ecology of the species in the context of the various vegetation-land patterns that exist is known, then it is possible to make an educated guess as to what is *likely* to happen in the future. Theory may suggest that generally the upland aspen stands in the boreal forest will be succeeded by white spruce, but in the context of specific geographic place this may not be so. For example, in north-

central Saskatchewan, aspen on low, narrow till ridges between intervening depressions stocked to black spruce apparently develops toward the black spruce cover type. But on very dry sands of the same region aspen stands may show gradual change to a self-perpetuating pine forest, or to white spruce – pine on south-facing slopes and to black spruce – birch on north-facing slopes. The concept of multidirectional succession is of course recognized by many students of vegetation.

Discussion

There are two levels at which forests can profitably be subjected to scientific study. First there is the level of the tree species, where individuals that appear to be similar are made the focus of physiological, ecological, and genetical study. The second is the geographic level where attention is focused on forest-land entities (ecosystems) as they exist at particular places. Here the forest trees and associated minor vegetation are seen in the context of a specific terrain and surroundings, and study is directed to the processes going on within units of earth-area where forest, soil (in the broad sense), and climate interact. Both levels are important in providing useful knowledge, and both are interrelated. The ecological study of trees, for example, shows how they may adapt to conditions of specific geographic place, while on the other hand the study of process in geographic forest-land units reveals the environmental tolerances of the participating plants.

As a biological science, forestry has found at least part of its theoretical foundation in plant sociology (known also as "ecology" in North America), and the suppositions of the latter have frequently been reflected in the former. A basic premise, and I suggest a serious source of error, has been the notion that a third level of study exists, of equal status with the individual of the species and the geographic ecosystem—namely the plant community. From this it has been supposed that a formal science of plant communities (vegetation), complete with universally valid principles, is possible outside the context of geographic place.

The status of the community as an object of study has been argued for many years. It is generally agreed that communities are not organisms, and that their internal integration and organization is loose and variable. In this there is the hint that we are dealing with a nonsystem, and the suspicion is reinforced when an attempt is made to apply the scientific viewpoints of physiology and ecology to any selected community. It is apparent that for an individual plant or animal the viewpoints have meaning, likewise for any geographic ecosystem as a volume of earthly space with its aggregate of organisms. But a forest stand (community of trees) has neither physiology nor ecology, except as it is (1) broken down into its individual trees, or (2) placed within the physical ecosystem of which it is part. The *forest community* is a botanical concept; it is a compositional (nonstructural) element of the objective level-of-integration: the geographic ecosystem (Rowe 1961).

There are important implications in the assertion that forest and other vegetational communities ought to be studied in their geographic contexts, as parts of ecosystems rather than as things-in-themselves. Most obvious is the one stressed earlier, that concepts such as "competition", "succession",

and "climax" can be given specific meaning only within a framework of geographic environment. By logical extension we are led to question the proposal (particularly from the lips of ecologists) that vegetation and other communities be set at the center of scientific study, and described, classified, and studied in terms of their attributes alone. Such a procedure neglects the objective system in favor of a compositional component.

Furthermore, a skeptical eye can be cast on the frequently expressed (though contradictory) notions that, for the derivation of basic principles, vegetation ought to be studied in its most complex forms (in the tropics) or in its simplest forms (in deserts and tundra). Study of vegetation in one region need not be productive of useful generalizations for other regions. In fact it is doubtful whether there can be a systematic science of ecosystems, let alone of vegetation communities, with formal laws and general principles, for this is not in the nature of geographic phenomena. Our world is unique, and the remark is appropriate that geography, like history, does not repeat itself (although geographers, and historians, frequently repeat one another). True, geographic phenomena—mountains, lakes, forest-land—are repetitive in their general outlines and can to a point be studied systematically, but geographic uniqueness limits the scope of generalizations about them all. Only the broadest and least useful principles transcend the necessary confines of a geographic framework. Working principles, formed at the local or regional levels, cannot be expected to have world-wide significance.

Therefore, in addition to the question of the general validity of the succession-to-climax concept is the question as to the justification of transferring generalities about vegetation from region to region. On theoretical grounds it does not seem likely that vegetational concepts from the context of mid-Europe will fit the forests of the Canadian boreal region. The concept of "climax" as a self-perpetuating forest of maximum productivity *may* agree with the facts in some temperate, leaf-tree ecosystems, but it may be unrealistic in the tropics, or in those parts of the northern needleleaf forest where the prevailing species are adapted to establishment in mineral soil, rather than in humus, and are not particularly tolerant of shade. Thus in the forests of Quebec, where black spruce and balsam fir are dominants, Linteau (1955) has reported that the even-aged black spruce stands which follow fire are superior in productivity to the older, uneven-aged stands that succeed them. The same can of course be said for the pioneer stands of Douglas fir on the Pacific Coast. Apparently there are parallels in some of the boreal forests of the Scandinavian countries, and as a result a strong case can be made for even-aged management through clear-cutting of the forests (Zivnuska 1959).

In some parts of the world the "selection forest" may be a silvicultural ideal, but this need not be universally true. Similarly, one can justifiably question a number of other propositions, often assumed to be valid everywhere but which in some geographic contexts may not be: for example, that mixed forests are necessarily healthier than single species forests, that nutrient regime of forest soils increases with age, that monoculture of conifers degrades the soil, that burning and clear-cutting of the forest degrades site and lowers potential for productivity. Universal rules ought to be suspect in forest science; the need is for regional guides derived inductively.

Final Remarks

In the contemporary world all nations share a vital concern: the rational use of land resources. Two different approaches are dictated by age, size, and population of country (Journaux and Dewolf 1959). In the newer parts of countries such as Canada where population is small and area large, it is a question of evaluating the land, of preventing unconsidered exploitation, and of deciding on the best possible present and future land use. The problems are different in long-occupied countries such as those of western Europe. There, the need is to rehabilitate impoverished and degraded land, to restore vegetative cover and soil fertility, to change the old routine cultural practices so that environmental conditions will be increasingly ameliorated. It is understandable that in the latter circumstances there should be an idealization of the primeval natural state, the "way of Nature".

The duplicity of nature is proverbial and anyone seeking to understand "her ways" is likely to find what he expects to find. Doubtless the primeval state is a good model to hold up to countries convalescing after destructive exploitation; in new countries it may be less useful for it poses no challenge. Too great a reverence for "Nature", coupled with intuitive discovery of her "laws of development", may impose an excessive conservatism on silviculture and on soil science, just as the parallel belief in fictional laws of historical necessity has paralyzed political action. Neither Nature nor History are sources of inflexible permissive laws, and it is a good premise that whatever is or has been can be improved on. We have, for example, strong arguments from such men as Nikiforoff (1959) and Hiley (1959) that the most efficient way to manage the land for increased production is to replace wild with artificial vegetation. However, the need for careful study and appraisal of any managerial innovations cannot be overstressed.

Returning to Plochmann's study, it suffers much from the dominant *a priori* element. The prefabricated picture of forest development which he has projected on the scene seems not to be harmonious with this particular geographic region. Examined in the context of what is known of the boreal forests of western Canada, and in the light of recent ecological thought, his report can only be judged as of small significance to Canadian foresters.

References

HILEY, W. E. 1959. Conifers: South African methods of cultivation. Faber & Faber, London.

JONES, E. W. 1945. Structure and reproduction of the virgin forest of the north temperate zone. New Phytologist, **44**, 130–148.

JONES, E. W. 1959. Review of article by R. Plochmann: Bestockungsaufbau und Baumartenwandel nordischer Urwälder. J. Ecol. **47**(2), 523–524.

JOURNAUX, A. and DEWOLF, Y. 1959. Essai d'une définition de régions géopédologiques. Cahiers de Géograph. de Québec No. 6. pp. 53–55.

KÖSTLER, J. 1956. Silviculture. *Translated from* Waldbau (P. Parey, Hamburg) *by* M. L. Anderson. Oliver and Boyd, London.

KÖSTLER, J. 1959. Sapinières du Saint-Laurent. J. forestier suisse, **110**(3), 149–162.

LAMPRECHT, H. 1954. Ueber Strukturuntersuchungen im Tropenwald. Z. Weltforstwirtsch. **17**(5), 161–168.

LEIBUNDGUT, H. 1959. Uber Zweck und Methodik der Struktur- und Zuwachs-analyse von Urwäldern. Schweiz. Z. Forstw. **110**(3), 111–124.

LINTEAU, A. 1955. Forest site classification of the northeastern coniferous section, Boreal Forest Region, Quebec. Can. Dept. Northern Affairs Natl. Resources, Forestry Branch Bull. 118.

MOSS, E. H. 1953. Forest communities in Northwestern Alberta. Can. J. Botany, **31**, 212–252.

MOSS, E. H. 1955. The vegetation of Alberta. Botanical Rev. **21**, 493–567.

NIKIFOROFF, C. C. 1959. Reappraisal of the soil. Science, **129**(3343), 186–196.

PLOCHMANN, R. 1956. Bestockungsaufbau und Baumartenwandel nordischer Urwälder. Beih. Forstwiss. Centr. Forstwiss. Forsch. **6**, 1–96.

PEACE, T. R. 1960. The dangerous concept of the natural forest. Advance. of Sci. **68**, 1–8.

ROWE, J. S. 1955. Factors influencing white spruce reproduction in Manitoba and Saskatchewan, Can. Dept. Northern Affairs Natl. Resources, Forest Research Div., Tech. Note No. 3.

ROWE, J. S. 1961. The level-of-integration concept and ecology. Ecology, **42**, 420–427.

VEZINA, P. E. 1959. Contribution à l'étude des forêts vierges comme base pour le développement d'une silviculture plus près de la nature. Essai d'application aux forêts résineuses de l'Est canadien. J. forestier suisse, **110**(3), 135–149.

WHITTAKER, R. H. 1953. A consideration of the climax theory: The climax as a population and pattern. Ecol. Monographs, **23**, 41–78.

ZIVNUSKA, J. A. 1959. Private forestry in Norway—a case study in small woodland management and policy. Forest Sci. Monograph, **1**, 1–49.

EFFECT OF RUST-INHIBITING COMPOUNDS ON THE METABOLISM OF WHEAT LEAVES[1]

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Abstract

Healthy leaves of Little Club wheat (*Triticum compactum* Host.) and leaves infected by leaf rust (*Puccinia recondita* Rob. ex. Desm. race 5) were detached and floated for 3 days on solutions containing 10 p.p.m. canavanine, 0.1% azathymine, 0.125% dulcitol, and 0.1% methionine. Leaf samples were fed with uniformly labelled glucose for 24 hours, fractionated, and the C^{14}-content of each fraction was determined. Compared with controls, canavanine slightly reduced the amount of C^{14} in amino acids, nucleic acids, and proteins while 6-azathymine markedly reduced the amount of C^{14} in nucleic acids and proteins. Treatment with dulcitol resulted in a lower recovery of C^{14} from all fractions other than sugars while methionine had little effect on the distribution of C^{14} from glucose. Quantitative analyses showed a marked increase in amino acids after canavanine, 6-azathymine, and methionine treatments and a reduced level of nucleic acids after treatment with canavanine and azathymine.

Canavanine had no effect on the incorporation of arginine and glutamic acid into leaf proteins while 6-azathymine inhibited the incorporation of adenine into nucleic acids. It was not possible to relate the changes that occurred following these treatments to the *in vivo* metabolism of the rust fungus.

Introduction

It was recently shown that a variety of compounds, including metabolites and antimetabolites, can inhibit rust development on detached wheat leaves (13). Inhibition of facultative parasites on hosts supplied with amino acids had been previously reported by van Andel (1). Various compounds markedly influence host metabolism and the host–parasite relationship. Fuchs and his co-workers have studied a number of such compounds with several hosts and parasites and have carried out extensive investigations on the effects of these compounds on host metabolism (3, 4, 5, 6, 7, 9, see also 12). If a particular compound were to inhibit the parasite directly, without altering host metabolism, it would be useful in studying the *in vivo* metabolism of an obligate parasite. This investigation was undertaken to determine the extent to which representative rust-inhibitory compounds alter host metabolism and to evaluate the usefulness of this experimental approach to the study of obligate parasitism.

Materials and Methods

First leaves of Little Club wheat (*Triticum compactum* Host.) bearing 2-day-old infections of leaf rust (*Puccinia recondita* Rob. ex Desm. race 5), and comparable noninfected leaves, were detached and floated for 3 days on solutions of 10 p.p.m. canavanine, 0.1% 6-azathymine, 0.125% dulcitol, and 0.1% methionine. All solutions contained 32 p.p.m. benzimidazole (14); leaves floated on 32 p.p.m. benzimidazole alone served as controls.

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After 3 days on the test solutions, the leaves were removed, blotted dry, and cut to an over-all length of 3 inches. Samples of 12 leaves each were allowed to take up 5 μc of uniformly labelled glucose-C^{14} through their cut basal ends. All samples were kept in a growth chamber at 21° C under continuous illumination at a light intensity of 700 ft-c. After uptake of the glucose-C^{14} was nearly complete, small aliquots of the appropriate inhibitor solutions were added repeatedly before addition of sufficient solution to last overnight.

After 24 hours, the leaves were removed from the solutions, 1 cm from the basal end of each leaf was discarded, and the fresh weight of each sample was determined. The leaves were then cut into approximately 0.5-cm pieces and dropped immediately into liquid nitrogen. After evaporation of the nitrogen, solid CO_2 was added; the beakers were then covered with aluminum foil and stored at $-29°$ C.

The leaf samples were fractionated following the procedures described by Roberts *et al.* (11). These fractions are not pure but are designated according to the materials which would predominate in each fraction. The frozen leaf sections were dropped into boiling 95% ethanol and boiled for 5 minutes. After the ethanol was decanted, the residue was subjected to two half-hour extractions with boiling 80% ethanol and one half-hour extraction with boiling 40% ethanol. All ethanol extracts were combined and the alcohol was removed by evaporation. The aqueous extract, after removal of chlorophyll by liquid–liquid extraction with chloroform, was passed through Dowex 50 and Dowex 1 ion-exchange columns, yielding organic acid, sugar, and amino acid fractions (16).

The residue after alcohol extraction was defatted with alcohol–ether (3:1). This extract was termed the lipid fraction. The defatted residue was extracted with 0.5 *N* perchloric acid for 20 minutes at 100° C, filtered, and the residue repeatedly washed with 0.5 *N* perchloric acid. The perchloric-acid-soluble portion was brought to pH 7.0 with 5 *N* KOH and the precipitated $KClO_4$ was removed.

The residue after perchloric-acid extraction was hydrolyzed with 6 *N* HCl for 12 hours at 120° C. The hydrolyzate was filtered and the filtrate was evaporated repeatedly until excess HCl was removed. It was then passed through a Dowex 50 column yielding an effluent termed the cellulose fraction and the eluate or protein fraction.

For determination of C^{14}, each fraction was made up to 2 ml and three 25-μl aliquots were plated uniformly onto planchets. The activity was determined with a gas flow counter fitted with a micromil end window.

Chlorophyll was determined spectrophotometrically at 675 mμ (2). Absorbency at 260 mμ with 0.15 *N* NH_4OH as the solvent was used to give an estimate of nucleic acids. Sugars were determined by an anthrone method (17) while amino acid and protein nitrogen were determined according to Umbreit *et al.* (15). Organic acid content was not measured.

A few leaves were kept on each experimental solution and examined for rust development 9 days after inoculation. Rust development was heavy on the leaves floated on benzimidazole alone and slight to moderate on those floated on dulcitol. There was no sporulation or other visible evidence of rust development on azathymine, canavanine, or methionine.

Results

It can readily be seen from Table I that the distribution of C^{14} from uniformly labelled glucose was markedly different in the control (BN and BR) and treated leaves. The leaves had been detached and floated on the test solutions for 3 days before feeding glucose-C^{14}, and the distribution of C^{14} would be influenced by altered pool sizes which would tend to exaggerate a metabolic defect. A large increase in free amino acids would result in a lower recovery of C^{14} from the protein fraction. Quantitative determinations of some fractions are given in Table II, while the relative specific activities of these fractions are shown in Table III. The expression of the C^{14} distribution as the percentage of total counts in the sample (Table I) is the most satisfactory method for this type of experiment.

Canavanine treatment resulted in a lower recovery of C^{14} from amino acids, proteins, and nucleic acids, but the differences from control leaves were not striking (Table I). With azathymine, C^{14} in proteins and nucleic acids was markedly lower than in control leaves. Dulcitol appeared to inhibit carbohydrate breakdown in wheat leaves since much of the activity was recovered in the carbohydrate fraction, with a reduced level of C^{14} in all other fractions. The analyses shown in Table II suggest that this effect is not due to an increased pool of endogenous carbohydrate. The concentration of dulcitol used did not completely inhibit rust development, and considerable breakdown of sugars occurred in the dulcitol-treated leaves which were infected with rust (DR, Tables I and II). In general, in rust-infected leaves kept on these solutions, the distribution of C^{14} more nearly approached the values obtained from untreated leaves. Methionine had little effect on host metabolism although incorporation of C^{14} into chlorophyll was depressed. These leaves contained considerable amounts of methionine sulphoxide as well as a higher level of methionine.

It can be seen from Table II that the most striking effects of these treatments were on the levels of amino acids and nucleic acids. Treatments with canavanine, azathymine, and methionine increased amino acid levels while nucleic acids decreased in leaves treated with canavanine and azathymine.

The nucleic acid levels in rust-infected untreated leaves were unexpectedly low (Table II, BR). It should be emphasized that these experiments were carried out with detached leaves, and that after detachment, changes in the levels of some fractions (e.g. compounds containing nitrogen) can occur only at the expense of other fractions. Changes in a few fractions were investigated in leaves of Little Club wheat detached 1 day after inoculation and floated on 32 p.p.m. benzimidazole. At 2, 4, 6, and 8 days after inoculation, samples of 24 leaves were fed uniformly labelled glucose-C^{14} for 5 hours, fractionated, and the fractions were counted and analyzed for content of amino acids, proteins, and nucleic acids. Under these experimental conditions, nucleic acid levels increased after infection, amino acid levels declined, while protein levels (apart from an anomalous result for 6 days after inoculation) remained relatively constant (Table IV). However, the specific-activity data of Table V indicate a higher rate of protein turnover in rusted than in healthy tissue.

Canavanine and azathymine would be expected to have a fairly specific effect on the synthesis of proteins and nucleic acids, respectively. Primary

TABLE I

Amount of C^{14} (expressed as percentage of total radioactivity) in various fractions taken from detached healthy and rusted leaves following treatment with rust inhibitors

Fraction	Leaf material:									
	BN	BR	CN	CR	AN	AR	DN	DR	MN	MR
Lipid	0.03	1.3	0.04	0.05	0.03	0.04	0.00	0.10	0.03	0.04
Chlorophyll	3.4	3.8	2.8	3.4	2.8	1.8	1.6	2.9	1.4	1.6
Amino acids	7.9	2.3	6.1	5.8	6.8	9.9	2.5	3.4	9.6	10.9
Sugars	51.6	32.8	60.5	55.9	71.0	62.8	73.4	57.0	46.5	48.2
Organic acids	16.5	29.3	16.2	14.6	10.9	14.2	10.4	16.2	19.3	18.6
Nucleic acids	10.8	19.2	7.7	11.6	3.8	7.7	8.0	13.4	14.7	13.0
Cellulose	0.4	0.6	0.3	0.8	0.1	0.1	0.1	0.2	0.3	0.5
Protein	7.8	7.4	5.3	6.7	3.8	2.5	3.2	5.3	6.8	5.8
Waste fract.*	0.7	0.7	0.4	0.4	0.4	0.3	0.2	0.3	0.3	0.5
Waste fract.†	0.9	2.5	0.6	0.8	0.4	0.7	0.6	1.2	1.1	0.9
Total (%)	100.0	99.9	99.9	100.1	100.0	100.0	100.0	100.0	100.0	100.0

NOTE: The fractions shown in this and subsequent tables are not pure but are designated according to the predominant compounds.
B = floated on benzimidazole, 32 p.p.m.
C = floated on benzimidazole, 32 p.p.m., containing 10 p.p.m. canavanine.
A = floated on benzimidazole, 32 p.p.m., containing 0.1% 6-azathymine.
D = floated on benzimidazole, 32 p.p.m., containing 0.125% dulcitol.
M = floated on benzimidazole, 32 p.p.m., containing 0.1% methionine.
N = noninoculated (first leaves of Little Club).
R = rusted (first leaves of Little Club inoculated with race 5 of leaf rust, harvested on 6th day after inoculation).
*Insoluble material after concentration of alcohol-soluble fraction.
†Insoluble material after protein hydrolysis, mainly melanins.

TABLE II

Chlorophyll, sugar, amino acid, protein, and nucleic acid levels in detached healthy and rusted wheat leaves following treatment with various rust inhibitors

Fraction	Leaf material:									
	BN	BR	CN	CR	AN	AR	DN	DR	MN	MR
Chlorophyll*	0.96	0.96	1.06	0.85	1.02	0.93	1.24	0.90	0.90	0.79
Sugars*	8.16	3.65	9.61	6.20	4.85	4.58	6.04	4.64	7.89	5.75
Amino acids†	0.81	0.72	1.48	1.45	1.79	2.15	0.89	0.87	2.78	3.12
Protein†	6.04	7.04	5.60	6.13	6.44	6.43	8.54	8.64	6.81	6.18
Nucleic acids (arbitrary units)‡	24	13	14	23	13	27	27	23	29	26

*Mg/g fresh weight.
†Mg N/g fresh weight.
‡Based on optical density of solution at 270 mμ.

TABLE III

Specific activities of chlorophyll, sugar, amino acid, protein, and nucleic acid fractions of detached wheat leaves following treatment with various rust inhibitors

Fraction	Leaf material:									
	BN	BR	CN	CR	AN	AR	DN	DR	MN	MR
Chlorophyll*	4.5	4.4	4.5	4.8	4.6	3.2	1.9	3.8	1.6	2.5
Sugars*	7.9	10.7	10.6	10.7	24.5	23.3	17.7	14.7	6.1	10.2
Amino acids†	12.4	3.7	6.9	4.8	6.4	7.8	4.2	4.7	3.6	4.3
Protein†	1.6	1.2	1.6	1.3	1.0	0.7	0.6	0.7	1.0	1.1
Nucleic acid‡	5.7	15.8	9.3	5.9	4.6	4.9	4.3	8.5	5.2	6.0

*c.p.m./mg ($\times 10^6$).
†c.p.m./mg N ($\times 10^6$).
‡c.p.m./unit optical density of solution at 270 mμ ($\times 10^8$).

TABLE IV

Levels of amino acids, proteins, and nucleic acids in leaf samples collected at various times after inoculation with rust

Fraction	Leaf material: 2BN	2BR	4BN	4BR	6BN	6BR	8BN	8BR	6N	6R
Amino acids*	1.58	1.35	1.82	1.03	2.02	1.31	2.00	1.12	2.10	6.53
Protein*	7.86	7.10	7.73	7.31	7.83	10.17	6.48	7.10	6.09	5.16
Nucleic acids†	10	7	—	16	—	24	15	25	8	16

NOTE: BN = nonrusted leaves floated on benzimidazole solution (32 p.p.m.).
BR = rusted leaves floated on benzimidazole solution (32 p.p.m.); leaf rust, race 5a.
2, 4, 6, 8 = harvested on the 2nd, 4th, 6th, or 8th day after inoculation.
6N, 6R = nonrusted or rusted attached leaves, respectively, harvested on 6th day after inoculation.
*Mg N/g fresh weight.
†Arbitrary units based on optical density of solution at 270 mμ.

TABLE V

Specific activities of amino acid, protein, and nucleic acid fractions in leaf samples collected at varying times after inoculation with rust

Fraction	Leaf material: 2BN	2BR	4BN	4BR	6BN	6BR	8BN	8BR	6N	6R
Amino acids*	9.6	24.8	18.5	12.2	12.6	16.3	14.4	17.3	13.6	3.5
Protein*	5.4	4.9	1.8	4.0	1.7	3.9	1.4	3.0	6.4	9.6
Nucleic acids†	12.1	28.4	—	22.3	—	29.7	11.0	19.6	32.0	37.0

*c.p.m./mg N ($\times 10^6$).
†c.p.m./unit optical density of solution at 270 mμ ($\times 10^3$).

leaves of Little Club wheat were detached, floated on canavanine and azathymine solutions for 3 days, then fed C^{14}-labelled compounds for 5 hours. The amount of C^{14} incorporated into proteins and nucleic acids as well as unincorporated or soluble C^{14} was determined and the ratios of insoluble to soluble C^{14} are shown in Table VI.

Canavanine had no effect on the incorporation of arginine or glutamic acid into protein while azathymine reduced incorporation of adenine into nucleic acids. In a further experiment with azathymine, adenine-C^{14} was fed to treated and control leaves and the ratios of C^{14} in nucleic acids to C^{14} in the soluble fraction at the end of 5 and 23 hours were determined (Table VII).

Increasing the incubation time did not alter the azathymine effect since after 23 hours the ratio increased 3.5-fold in both control and treated leaves.

TABLE VI

Incorporation of C^{14}-labelled compounds into protein and nucleic acid fractions of wheat leaves following treatment with inhibitors

Treatment	C^{14} compound added	Ratio $\frac{\text{insoluble } C^{14}}{\text{soluble } C^{14}}$
Control	Glutamic acid	0.111
	Arginine	0.012
Canavanine	Glutamic acid	0.116
	Arginine	0.011
Control	Adenine	0.095
Azathymine	Adenine	0.064

TABLE VII

Effect of azathymine on the incorporation of adenine into the nucleic acid fraction of wheat leaves

Treatment	Time	Ratio $\frac{C^{14} \text{ in nucleic acids}}{C^{14} \text{ in soluble fraction}}$
Control	5 hours	0.053
Azathymine	5 hours	0.034
Control	23 hours	0.185
Azathymine	23 hours	0.120

Discussion

At present it is not known to what extent the rust fungus depends on the enzymatic systems of the host. Different organisms exhibit varying degrees of sensitivity to inhibitors, and a compound which does not inhibit host metabolism but does inhibit rust growth should give information on the *in vivo* metabolism of the parasite with respect to the particular sequence affected by that compound. Unfortunately, the results of the present study are difficult to interpret in these terms. While it is possible that the fungus is directly inhibited by these treatments, it was seen that canavanine, azathymine, and dulcitol had considerable effects on glucose metabolism in the host, and it is also possible that these effects on the host may account for the observed

inhibition of rust development. This is a recurring dilemma in studies on obligate parasites since fungal metabolism can be studied independently of the host only during spore germination. It is often assumed that a substance which does not inhibit spore germination and germ tube elongation but prevents growth of the pathogen on its host does so by altering host metabolism. However, this may not be a valid assumption. A compound which does not affect the physiology of spore germination may have profound effects on later development and differentiation of the pathogen within the host.

Considering the host–parasite association as an interacting complex, it is apparent that interference with carbohydrate, nucleic acid, or protein metabolism may be inhibitory to the complex. A number of studies have shown that amino acid analogues, including canavanine, are incorporated into specific proteins with concomitant impairment of function (8, 10). Excessive levels of methionine may similarly lead to synthesis of proteins with impaired function. Analogues such as azathymine, which affect nucleic acid metabolism, would be expected to interfere with protein synthesis, either through a general reduction of synthesis or through the production of abnormal proteins. It is apparent that more precise information on the action of these compounds in our system is required. This may be obtained by studying their effects on the activity of specific proteins whose concentrations change after infection, while histological methods may be useful in differentiating between effects on host and pathogen.

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References

1. ANDEL, O. M. VAN. 1958. Investigations on plant chemotherapy. II. Influence of amino acids on the relation plant–pathogen. Tijdschr. Plantenziekten, **64**, 307–327.
2. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1950. Official and tentative methods of analysis. 7th ed. Assoc. Official Agr. Chemists, Wash.
3. CHRISTIANSEN-WENIGER, E. 1955. Versuche zur stoffwechselphysiologischen Beeinflussung der Reaktion der Kartoffelknolle auf *Phytophthora infestans* de By. Phytopathol. Z. **25**, 153–180.
4. FUCHS, W. H. 1957. Weitere Beiträge zur pathologischen Physiologie der Pflanze. Proc. Intern. Congr. Crop Protect. 4th Congr., Hamburg. Vol. 1 (Braunschweig, 1959). pp. 101–104.
5. FUCHS, W. H. 1958. Zur Biochemie parasitärer Symbiosen. Angew. Botan. **32**, 221–227.
6. FUCHS, W. H. and BAUERMEISTER, R. 1958. Über die Wirkung von Thiosemicarbazid auf die Entwicklung von *Puccinia graminis tritici* auf Weizen. Naturwissenschaften, **45**, 343, 344.
7. HEITEFUSS, R. and FUCHS, W. H. 1960. Untersuchungen zur pathologischen Physiologie von *Peronospora parasitica* auf *Brassica oleracea*. Phytopathol. Z. **37**, 348–378.
8. KERRIDGE, D. 1960. The effect of inhibitors on the formation of flagella by *Salmonella typhimurium*. J. Gen. Microbiol. **33**, 519–538.
9. NIELSEN, J. 1960. Weitere Untersuchungen zur pathologischen Physiologie von *Peronospora parasitica* auf *Brassica oleracea*. Phytopathol. Z. **40**, 117–142.
10. RICHMOND, M. H. 1960. Immunological properties of exopenicillinase synthesized by *Bacillus cereus* 569/H in the presence of amino acid analogues. Biochem. J. **77**, 112–121.
11. ROBERTS, R. B., COWIE, D. B., ABELSON, P. H., BOLTON, E. T., and BRITTEN, R. J. 1955. Studies of biosynthesis in *Escherichia coli*. Carnegie Inst. Wash. Publ. 607, Wash., D.C.
12. ROHRINGER, R. 1959. Allgemeine Pflanzenpathologie. Fortschr. Botan. **21**, 361–384.

13. SAMBORSKI, D. J. and FORSYTH, F. R. 1960. Inhibition of rust development on detached wheat leaves by metabolites, antimetabolites, and enzyme poisons. Can. J. Botany, **38**, 467–476.
14. SAMBORSKI, D. J., FORSYTH, F. R., and PERSON, C. 1958. Metabolic changes in detached wheat leaves floated on benzimidazole and the effect of these changes on rust reaction. Can. J. Botany, **36**, 591–601.
15. UMBREIT, W. W., BURRIS, R. H., and STAUFFER, J. F. 1949. Manometric techniques and tissue metabolism. Burgess, Minneapolis. p. 161.
16. WANG, D. 1960. An ion-exchange resin method for the fractionation of alcoholic plant extracts. Nature, **186**, 326–327.
17. YEMM, E. W. and WILLIS, J. A. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. **57**, 508–514.

EFFECT OF BENZIMIDAZOLE ANALOGUES ON STEM RUST AND CHLOROPHYLL METABOLISM[1]

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Abstract

A study of the structural specificity of benzimidazole in relation to its effect on stem rust development and chlorophyll metabolism was made to obtain information which may contribute to our understanding of the nature of rust resistance as well as the physiology of detached leaves of Khapli wheat. Evidence suggests that benzimidazole will lose its activity if any member of the elements in its imidazole ring and probably also in its benzene ring is replaced by a different element, such as carbon, oxygen, nitrogen, or sulphur. Furthermore, compounds in which the methyl, nitro, or other group is introduced into the molecule of benzimidazole are either phytotoxic or antagonistically active to their parent compound.

Analogues which were antagonistically active to benzimidazole displayed a similar effect on kinetin. There is strong evidence that benzimidazole and kinetin may play a similar role in maintaining the rust resistance and the normal physiology of detached wheat leaves.

Introduction

The question of why some wheat plants are resistant to a given rust race while others are not has been studied by numerous investigators. Normally, in a study of this nature, two or more varieties of wheat possessing different degrees of resistance or susceptibility are used with a given rust race. In the writers' opinion this is not a satisfactory system to use because fundamental biochemical and physiological differences exist between varieties. It would be better to use one variety only and two rust races, with the plant resistant to one race and susceptible to the other. With such a system at least the biochemical and physiological differences that exist between varieties can be eliminated. In our work a detached leaf system was adopted. This is a relatively good system, but by no means ideal because it consists of an isolated organ of a whole plant. It does, however, offer many biological and technical advantages.

Leaves of Khapli wheat normally resistant to race 15B-1 of *Puccinia graminis tritici* become susceptible and chlorotic when detached and floated on water. This breakdown of resistance and green pigment can be reversed by floating the detached leaves on a solution of benzimidazole (5, 7). Since resistance or susceptibility can be altered with ease, this system is quite a satisfactory one for studying the nature of rust resistance as well as the physiology of detached wheat leaves. Recently, Wang *et al.* (8, 9) have shown that benzimidazole maintains the normal rate of chlorophyll biosynthesis and prevents the destruction of the green pigment in light and in darkness. It has also been reported (3) that benzimidazole maintains the insoluble nitrogen

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content of detached leaves of Little Club wheat at a level higher than the water control. However, the mode of action of benzimidazole and its structural specificity with respect to its ability to maintain rust resistance and normal chlorophyll metabolism in detached leaves remains obscure. In an attempt to elucidate this point a study was made of the effect of its analogues on the development of rust, as well as on their antagonistic action to benzimidazole with respect to the type of rust reaction and the maintenance of chlorophyll in detached leaves of Khapli wheat. The present paper deals with some of the results observed from this study.

Materials and Methods

The material, the method of inoculation and treatment, and experimental conditions adopted in this study are similar to those described previously (7). In experiments where the antagonistic action of benzimidazole analogues or other agents were studied, all solutions contained 50 p.p.m. of benzimidazole in addition to the compound under investigation. Estimates of rust reaction, chlorosis, and phytotoxicity were determined visually.

Results and Discussion

A number of benzimidazole analogues were tested and their effect on the development of rust and chlorosis in detached Khapli leaves is given in Table I. Only 5 out of 28 compounds investigated were active in preventing rust from developing. Among these five compounds, kinetin was the only one which showed a preservative effect on the green pigment of the leaves. This effect has also been reported by other workers (3, 4). The effective concentration of kinetin was much lower than that of benzimidazole. It was observed that kinetin and benzimidazole have similar effects in many ways. There may be a point of difference, however, in that benzimidazole appears to be more selective for monocotyledons than kinetin and in our experiments (unpublished) had no effect on the retention of the green pigment in the leaf tissue of bean, sunflower, and tobacco. Recently, Osborne and McCalla (2) have also shown that kinetin is active in the retention of chlorophyll in disks of senescing *Xanthium* leaves, whereas benzimidazole has only a slight effect. Moreover, kinetin has been reported (1) to inhibit elongation of etiolated pea stems, whereas benzimidazole had no effect in the presence of indoleacetic acid and sucrose. Many of the analogues that showed phytotoxicity varied only in degree. On the other hand, a number of them did not have any apparent effect on chlorophyll metabolism, i.e. the rate of chlorosis development in the treated leaves was similar to that of the water control. Some analogues showed no effect on rust development within the range of their nonphytotoxic concentrations.

The antagonistic effect of benzimidazole analogues towards the effect of benzimidazole on the development of rust and chlorosis in detached wheat leaves is presented in Table II. Among these compounds, adenine, 2-chlorobenzothiazole, benzotriazole, xanthine, 8-chloroxanthine, 8-azaxanthine, and 8-azaguanine were most effective in reversing the rust-inhibitory action of benzimidazole. These analogues, however, showed little or no antagonistic activity to benzimidazole in its effect on chlorophyll metabolism. Benzo-

TABLE I

Effect of benzimidazole and its analogues on the development of rust and chlorosis in detached leaves of Khapli wheat

Benzimidazole and its analogues	Development of rust and chlorosis* at various p.p.m. concentrations of test compound															
	5		10		20		30		40		50		60		80		100	
	R†	C†	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C
Benzimidazole	5	5	5	5	4	2	2	1	0	0	0	0	0	0	0	0	0	0
Benzoxazole	—	—	—	—	5	5	5	5	5	5	5	5	—	—	—	—	—	—
Benzotriazole	—	—	—	—	5	5	5	5	5	5	5	5	—	—	—	—	—	—
Benzothiazole	—	—	—	—	5	5	5	5	5	4	—	—	—	—	—	—	—	—
Benzimidazolethiol	—	—	—	—	—	—	—	—	5	5	—	—	—	—	—	—	—	—
Benzoxazolone	—	—	0	3	—	—	0	3	—	—	0	3	0	3	Toxic		Toxic	
Quinoxaline	—	—	—	—	4	4	—	—	—	—	2	4	—	—	1	5	0	5
2-Chlorobenzothiazole	—	—	4	4	—	—	4	4	—	—	2	5	Toxic		Toxic		Toxic	
5-Chloro-2-methylbenzimidazole	—	—	—	—	0	5	—	—	—	—	Toxic		—	—	—	—	Toxic	
2-*o*-Hydroxy-phenylbenzimidazole	—	—	Toxic		Toxic		Toxic		—	—	—	—	Toxic		—	—	—	—
2-Aminobenzimidazole	—	—	5	4	5	4	3	4	—	—	2	5	1	5	0	5	§	5
6-Nitrobenzimidazole	—	—	3	4	2	4	0	5	—	—	0	5	—	—	Toxic		Toxic	
2-Methylbenzoxazole	—	—	Toxic		Toxic		Toxic		Toxic		Toxic		Toxic		Toxic		Toxic	
Indole	—	—	—	—	5	3	4	5	5	1	Toxic		—	—	—	—	Toxic	
Adenine	—	—	—	—	4	4	—	—	—	—	3	4	—	—	—	—	2	4
Hypoxanthine	—	—	—	—	4	3	—	—	—	—	4	3	—	—	4	3	4	3
Xanthine	—	—	—	—	5	3	—	—	—	—	5	4	—	—	5	4	4	4
5,6-Dimethylbenzimidazole	3	4	3	4	3	4	2	5	—	—	—	—	—	—	—	—	—	—
4,5-Dicarboxylic acid imidazole	—	—	—	—	5	4	—	—	—	—	5	4	—	—	—	—	5	5
6-Mercaptopurine	—	—	—	—	3	4	—	—	—	—	3	4	—	—	2	4	2	4
Kinetin‡	5	1–5	5	1–4	3	1–3	0–1	0–1	0	0	0	0	0	0	—	—	—	—
5-Amino-2-benzimidazolethiol	—	—	4	4	—	—	5	4	—	—	4	4	—	—	4	4	4	4
5-Aminobenzimidazole	—	—	—	—	5	4	—	—	—	—	4	4	—	—	4	4	4	4
2-Benzoxazolethiol	—	—	0	4	0	5	Toxic		Toxic		Toxic		Toxic		Toxic		Toxic	
5-Chloro-2-methylbenzothiazole	—	—	5	5	5	5	5	5	5	5	5	5	—	—	—	—	—	—
8-Azaxanthine	—	—	—	—	5	3	—	—	—	—	5	3	—	—	5	4	4	4
8-Chloroxanthine	—	—	—	—	5	3	—	—	—	—	5	3	—	—	4	4	4	4
8-Azaguanine	—	—	—	—	5	3	—	—	—	—	5	4	—	—	5	4	4	4

*The arabic numerals are used to express the extensiveness of rust or chlorosis development in detached leaves: 5 is equivalent to that of water control and 0 to that of benzimidazole (50 p.p.m.) treated leaves.

†R represents the development of rust and C the development of chlorosis.

‡The concentrations of kinetin in increasing order are 0.2, 0.5, 0.8, 1.0, 2.0, 3.0, and 5.0 p.p.m. respectively.

§Necrotic area at the rust-infection site.

TABLE II

The antagonistic effect of benzimidazole analogues on benzimidazole with respect to the action of the latter on the development of rust and chlorosis in detached leaves of Khapli wheat

Analogue	Benzimidazole (p.p.m.)	Development of rust and chlorosis at various p.p.m. concentrations of test compound															
		10		20		30		40		50		60		80		100	
		R	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C
Benzotriazole	50	1	1	2	1	2	1	2	2	2	2	3	2	4	3	5	4
Benzoxazole	50	0	1	0	1	1	1	1	2	1	3	2	3	2	3	2	2
6-Nitrobenzimidazole	50	1	1	—	—	2	1	—	—	2	1	1	1	1	1	1	1
5-Amino-2-benzimidazolethiol	50	0	0	0	0	0	0	0	0	0	2	0	2	Toxic		Toxic	
5,6-Dimethylbenzimidazole	50	2	0	2	2	3	3	—	—	—	—	—	—	—	—	—	—
Indole	40	—	—	2	1	—	—	2	2	3	3	—	—	—	—	—	—
2-Chlorobenzothiazole	50	5	1	5	2	—	—	5	2	5	2	5	4	5	Toxic	Toxic	
Adenine	50	—	—	5	2	—	—	5	2	5	2	—	—	—	—	5	2
4,5-Dicarboxylic acid imidazole	50	—	—	2	2	—	—	—	—	3	3	—	—	—	—	3	3
Quinoxaline	50	—	—	0	1	—	—	—	—	0	3	—	—	0	4	0	4
2-Aminobenzimidazole	50	1	0	1	0	1	0	§	0	2§	1	3§	1	3§	1	4§	1
Hypoxanthine	50	—	—	0	2	—	—	—	—	§	2	—	—	—	—	2§	2
Benzoxazolone	50	0	0	—	—	0	0	—	—	0	0	—	—	0	0	Toxic	
2-Benzoxazolethiol	50	0	4	0	5	Toxic		Toxic		Toxic		Toxic		Toxic		Toxic	
5-Aminobenzimidazole	50	—	—	3	2	—	—	—	—	2	1	—	—	1	1	1	1
6-Mercaptopurine	50	—	—	2	2	—	—	—	—	2	2	—	—	2	2	2	2
Xanthine	50	—	—	3	1	—	—	—	—	4	2	—	—	4	2	4	2
8-Azaxanthine	50	—	—	4	2	—	—	—	—	4	2	—	—	4	2	4	2
8-Chloroxanthine	50	—	—	5	2	—	—	—	—	5	2	—	—	5	2	4	2
8-Azaguanine	50	—	—	5	2	—	—	—	—	5	2	—	—	5	2	4	2

§See Table I.

triazole gave erratic results. Apart from these antagonists, 2-aminobenzimidazole caused necrotic areas at the rust-infection sites. The most interesting and significant effect is the differential sensitivity of the leaf tissue towards this compound. This necrotic symptom resembles that of hypersensitivity. 2-Aminobenzimidazole in the presence of benzimidazole did not show any apparent deleterious effect on the green pigment of the detached leaves. In contrast, leaves treated with this compound alone developed chlorosis similar to the water control. With the highest concentration (100 p.p.m.) of 2-aminobenzimidazole, when used alone, necrotic areas occasionally appeared at the rust-infection site, but the extent of the necrosis was much less than when it was used in combination with benzimidazole. In view of the fact that, in the presence of benzimidazole, the host tissues around the rust pustules exhibited a differential sensitivity towards 2-aminobenzimidazole, further study along this line could yield useful information concerning the physiology of parasitism.

For purposes of studying the structural specificity problem, benzimidazole can be considered to consist of two separate parts, the benzene ring and the imidazole ring. Evidence suggests that benzimidazole will lose its activity if any element in the imidazole ring is replaced with a different element, such as carbon, oxygen, nitrogen, or sulphur. Examples are indole, benzoxazole, benzotriazole, and benzothiazole, respectively (Table I). This may be true also of its benzene ring. It is apparent from the data of Table I that both rings in the benzimidazole molecule are essential for the expression of its activity. Furthermore, an introduction of a methyl, amino, or other group into the molecule of benzimidazole will also alter its action in respect to rust development and to the chlorophyll metabolism in the detached wheat leaves. In view of the fact that none of the purine compounds tested other than kinetin had any benzimidazole-like activity, it is apparent that the activity of kinetin is very likely due to the combination of adenine and its furfuryl side chain rather than the purine ring alone.

Table III gives the results of the effect of compounds other than benzimidazole analogues on the action of benzimidazole and on rust development in detached leaves of Khapli wheat. Diphenylurea which was isolated from coconut milk by Shantz and Steward (6) as a growth factor showed no activity in our tests with detached leaves. Furthermore, this compound was extremely phytotoxic and caused chlorosis even when used with benzimidazole. Among the other compounds, furoic acid and choline effectively overcame the action of benzimidazole in rust development, but showed little effect in chlorophyll metabolism. The antagonistic activity of the compounds unrelated to benzimidazole is difficult to explain and the interpretation of its meaning must be left to further work.

Since kinetin and benzimidazole showed similarity in many of their activities in detached wheat leaves, a reversal of the kinetin activity may be expected with those compounds which were antagonistically active to benzimidazole. This assumption was found to be generally true (Table IV). In addition, kinetin and benzimidazole displayed a synergistic effect to each other. These results suggest that these two compounds may probably play a similar role in maintaining the rust resistance and the normal physiology of the detached wheat leaves. Comparative studies of their effects on carbon-14 dioxide

TABLE III

The effect of compounds other than benzimidazole analogues on the action of benzimidazole and on the development of rust and chlorosis in detached leaves of Khapli wheat

Compound	Benzimidazole (p.p.m.)	Development of rust and chlorosis at various p.p.m. concentrations of test compound													
		5		10		20		50		60		80		100	
		R	C	R	C	R	C	R	C	R	C	R	C	R	C
Furoic acid	—	—	—	—	—	2	5*	3	5*	—	—	—	—	3	5*
	50	—	—	—	—	2	0	—	—	—	—	—	—	3	1
Diphenylurea	—	—	—	Toxic		Toxic		Toxic		Toxic		Toxic		Toxic	
	50	—	—	—	—	—	—	2	5*	2	5*	2	5*	1	5*
Phenylurea	—	—	—	—	—	5	1	5	1†	—	—	—	—	—	—
	40	—	—	—	—	0	0	0	0†	1	0‡				
Choline	—	5	3	5	3	5	3	Toxic		Toxic		Toxic		Toxic	
	50	4	0	4	0	5	0	—	—	—	—	—	—	—	—
Furamide	—	—	—	—	—	5	1	5	1	—	—	—	—	5	1
Furamide+hypoxanthine (20 p.p.m.)	—	—	—	—	—	5	1	5	1	—	—	—	—	5	1
Furamide+hypoxanthine (50 p.p.m.)	—	—	—	—	—	5	1	—	—	—	—	—	—	5	1
Furamide+adenine (20 p.p.m.)	—	—	—	—	—	5	1	—	—	—	—	—	—	5	1
Furamide+adenine (50 p.p.m.)	—	—	—	—	—	5	1	—	—	—	—	—	—	5	1
Furoic acid + hypoxanthine (20 p.p.m.)	—	—	—	—	—	5	5	5	5	—	—	—	—	5	5
Furoic acid + hypoxanthine (50 p.p.m.)	—	—	—	—	—	5	5	5	5	—	—	—	—	5	5

*Phytotoxicity appeared.
†The concentration was 30 p.p.m.
‡The concentration was 40 p.p.m.

TABLE IV

The effect of compounds antagonistically active to benzimidazole on the activity of kinetin on the development of rust and chlorosis

Compound	Kinetin (p.p.m.)	Development of rust and chlorosis at various p.p.m. concentrations of test compound													
		0.5		1.0		5.0		10		20		40		50		100	
		R	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C
Adenine	0.5	1	0	2	1	—	—	3	1	3	1	4	1	—	—	—	—
	1.0	—	—	0	0	—	—	0	0	0	0	1	0	—	—	—	—
2-Chlorbenzothiazole	0.5	—	—	—	—	—	—	3	0	5	0	5	0	5	0	—	—
	1.0	—	—	—	—	0	0	0	0	1	0	3	0	4	0	—	—
Hypoxanthine	0.5	1	0	3	0	—	—	3	0	4	0	—	—	5	1	—	—
	1.0	2	0	3	0	—	—	3	0	4	0	—	—	5	1	—	—
Furoic acid	0.5	—	—	—	—	—	—	—	—	2	0	—	—	3	0	5	0
	1.0	—	—	—	—	—	—	—	—	4	0	—	—	4	0	4	0
Benzimidazole	0.5	—	—	—	—	0	0	0	0	0	0	—	—	0	0	—	—

assimilation, chlorophyll biosynthesis, nucleic acid metabolism, etc., have been made in this laboratory and will not be discussed here. Further study on the structural specificity of kinetin is undoubtedly needed.

References

1. FORSYTH, F. R. and SAMBORSKI, D. J. 1960. Effects of kinetin and benzimidazole on the growth of etiolated pea stems and barley coleoptiles. Can. J. Botany, **38**, 875–881.
2. OSBORNE, D. J. and MCCALLA, D. R. 1961. Rapid bioassey for kinetin and kinins using senescing leaf tissue. Plant Physiol. **36**, 219–221.
3. PERSON, C., SAMBORSKI, D. J., and FORSYTH, F. R. 1957. Effect of benzimidazole on detached wheat leaves. Nature, **180**, 1294–1295.
4. RICHMOND, A. E. and LANG, A. 1957. Effect of kinetin on protein content and survival of detached Xanthium leaves. Science, **125**, 650–651.
5. SAMBORSKI, D. J., FORSYTH, F. R., and PERSON, C. 1958. Metabolic changes in detached wheat leaves on benzimidazole and the effect of these changes on rust reaction. Can. J. Botany, **37**, 591–601.
6. SHANTZ, E. M. and STEWARD, F. C. 1952. Coconut milk factor. The growth-promoting substances in coconut milk. J. Am. Chem. Soc. **74**, 6133–6135.
7. WANG, D. 1959. Effect of benzimidazole, dimethylbenzimidazole, glucose, and metal ions on the development of *Puccinia graminis tritici* on detached leaves of Khapli wheat. Can. J. Botany, **37**, 239–244.
8. WANG, D. and WAYGOOD, E. R. 1959. Effect of benzimidazole and nickel on the chlorophyll metabolism of detached leaves of Khapli wheat. Can. J. Botany, **37**, 743–749.
9. WANG, D., HAO, M. S. H., and WAYGOOD, E. R. 1960. The effect of benzimidazole on the biosynthesis of chlorophyll in detached wheat leaves. Biochem. Biophys. Research Communs. **2**, 97–101.

THE ASSOCIATION BETWEEN AGE OF INFLORESCENCE AND NECTAR PRODUCTION IN THE LOW-BUSH BLUEBERRY VACCINIUM ANGUSTIFOLIUM[1]

G. W. Wood

Abstract

Nectar samples were harvested daily from exposed and caged portions of the low-bush blueberry, *Vaccinium angustifolium* (varieties *laevifolium* House and *nigrum* (Wood) Dole). Pollination activity on the exposed bloom had no significant effect on mean nectar volume and concentration but caused a slight reduction in the mean weight of nectar sugar. Nectar volume and weight of nectar sugar increased directly with the age of inflorescence in all clones.

Introduction

Studies on the pollination of low-bush blueberry in New Brunswick support the conclusions of Lee (4) that the blueberry plant is capable of setting and maturing fruit on a high percentage of its flowers, and that the honeybee is an efficient pollinator of this plant. The set in New Brunswick, however, is rarely higher than 50% regardless of the number of honeybee colonies used to supplement the native pollinators (9). One of the major factors contributing to this low set of fruit is the attraction of competitive bloom to the honeybee. Observations during the past 7 years have repeatedly shown that honeybees seek out the bloom of such plants as wild pear and apple and work them in preference to blueberry.

The present paper is based on part of a comprehensive study of the role of nectar in the relative attractiveness of the bloom of blueberry and associated plants. An understanding of the relationship between age of flower and nectar supply is prerequisite to the establishment of techniques for floral sampling. An extension of this study will include the attraction of insect pollinators to bloom of varying age.

Materials and Methods

Five clones of blueberry were selected and marked in each of two first-crop fields at Tower Hill, N.B. Eight of the clones were identified as *Vaccinium angustifolium* var. *laevifolium* House and two as *V. angustifolium* var. *nigrum* (Wood) Dole. Part of each clone was contained under a 6×3×3-ft screened cage in order to exclude insects, while the other part remained exposed. A sample of 50 open blossoms was taken from the exposed and caged parts of each clone through the period of bloom. Sampling was done between 8:00 and 10:00 a.m. daily. The first samples were taken on May 22 and the last on June 6. A few blossoms were open before May 22 but not sufficient to include a full sample. Some blossoms also opened after June 6 but most of the bloom had been open for at least 3 or 4 days at this time, and many of the corollas had fallen or were about to fall.

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Nectar was collected by the centrifugation method of Swanson and Shuel (6). Each floral sample was centrifuged for 5 minutes at approximately 1800 r.p.m. Concentration of solids in the nectar was measured with a hand refractometer. Because the present study is only concerned with comparative data, the product of volume, concentration, and specific gravity is expressed as weight of nectar sugar even though trace amounts of other solids are involved.

Results

In all clones sampled there was considerable variation in nectar volume and concentration between dates. Generally an increase in volume was compensated by a decreased concentration and vice versa. These variations were attributed to changes in temperature and humidity. Nectar volume ranged from unmeasurable amounts to 835 μl per 50 florets. Nectar concentration ranged from 3.0 to 58.5 per cent solids.

Clones of *V. angustifolium* var. *nigrum* did not differ significantly in any respect from those of the *laevifolium* variety, and the data from clones of both varieties have been grouped in all analyses. Unfortunately no clones of *V. myrtilloides* of sufficient size to permit extensive sampling were available for this study.

There was precipitation on two days during the period of bloom, 0.07 in. on the 4th day and 0.20 in. on the 10th day. Markedly increased volumes of nectar were harvested on the day following both periods of rainfall. In most samples, however, the increased volume was offset by a decrease in concentration.

There was no significant difference between the mean nectar volume and concentration from caged and exposed parts of blueberry clones. The difference between mean weights of nectar sugar was significant (Table I). Flowers which were exposed to insects contained less nectar sugar than those confined under cages.

Regardless of the influence of other factors or treatment, all clones showed the same relationship between age of flowering and mean nectar sugar content. Nectar volume and mean weight of nectar sugar increased directly with the age of inflorescence until about 10 days after the beginning of bloom (Fig. 1). From this period on the majority of corollas were dropping or withering, and

TABLE I

Volume and concentration of nectar and weight of nectar sugar per sample of 50 blossoms from caged and exposed portions of blueberry clones

	Number of samples	Caged portions		Exposed portions		Difference	*P*
		$\bar{x}$	S.E., $\bar{x}$, %	$\bar{x}$	S.E., $\bar{x}$, %		
Nectar volume (μl)	158	141.8	18.4	148.9	21.1	−7.1	0.70
Nectar concentration (per cent solids)	158	26.8	0.3	25.5	0.3	+1.3	0.40
Nectar sugar (mg)	158	27.3	0.5	22.3	0.3	+5.0	0.03

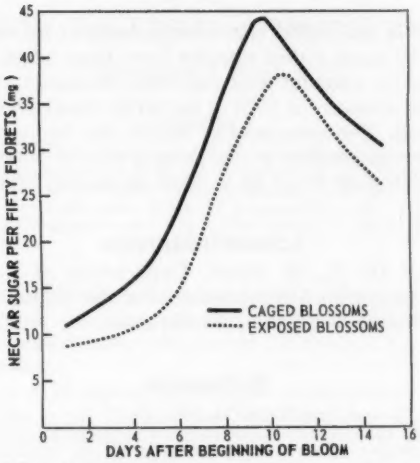

FIG. 1. The association between age of inflorescence and nectar sugar production in the low-bush blueberry.

the nectar sugar decreased in the flowers that were available for assay. The curves in Fig. 1 were both drawn from a three-period moving average of means of 10 readings on each date.

Discussion

The importance of nectar quantity and concentration in the attractiveness of flowers to bees, particularly honeybees, has been elucidated by many workers (2, 5, 7). Although data are not available on the relationship between nectar production in blueberry and bee visitation, it seems advisable to assume that a high nectar yield is a desirable quality.

During the present investigation an opportunity was afforded to obtain data on the effect of pollinator activity on attractiveness of blueberry bloom, as measured in terms of change in nectar and nectar sugar supply. There was no significant difference in nectar volume between exposed and caged blossoms, and although of statistical significance, the small difference in nectar sugar between exposed and caged blossoms would suggest that pollinator activity has a very minor influence on attractiveness.

The age of the flower has been shown to influence nectar secretion in several plants (1, 3, 8). In some plants, the old flowers secrete more nectar than the young flowers, in others the reverse relationship exists; some plants may have a high nectar yield in the old flowers but have a similar quantity of sugar to the young flowers. In blueberry, both nectar volume and nectar sugar content per clone increase through most of the bloom period. Therefore, if we assume a direct relationship between yield of nectar or nectar sugar and bee visitation, present evidence would show that the blueberry is more attractive to nectar-gathering insects towards the later part of the bloom period.

In summary, it is concluded that comparisons of nectar yield from blueberry should not be made unless samples have been taken from clones which have been in bloom for a similar period of time. The optimum time for sampling will vary with the seasons; in 1960 it occurred about 10 days after the first flowers had opened. The comparisons would also be more valid if samples were taken as close as possible to the same period of time. This would avert the influences which may occur as a result of rainfall or changes in weather conditions.

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References

1. Beutler, R. 1953. Nectar. Bee World, **34**, 106–116.
2. Butler, C. G. 1945. The influence of various physical and biological factors on the environment on honeybee activity. An examination of the relationship between activity and nectar concentration and abundance. J. Exptl. Biol. **21**, 5–12.
3. Free, J. B. 1960. The pollination of fruit trees. Bee World, **41**, 141–151.
4. Lee, W. R. 1958. Pollination studies on lowbush blueberries. J. Econ. Entomol. **51**, 544–545.
5. Pederson, M. W. 1953. Seed production in alfalfa as related to nectar production and honeybee visitation. Botan. Gaz. **115**(2), 129–138.
6. Swanson, C. A. and Shuel, R. G. 1953. The centrifuge method of measuring nectar yield. Plant Physiol. 25, 513–520.
7. Vansell, G. H. 1934. Relations between the nectar concentrations in fruit blossoms and the visits of honeybees. J. Econ. Entomol. **27**, 843–945.
8. Vansell, G. H., Watkins, W. G., and Bishop, R. K. 1942. Orange nectar and pollen in relation to bee activity. J. Econ. Entomol. **35**, 321–323.
9. Wood, G. W. 1961. The influence of honeybee pollination on fruit set of the low-bush blueberry. Can. J. Plant Sci. **41**, 332–335.

TYPIFICATION OF THE LINNAEAN SPECIES OF CARAGANA[1]

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Abstract

Four species of *Caragana* were described by Linnaeus under the genus *Robinia*. The following specimens in the Linnaean Herbarium of the Linnaean Society of London are selected as lectotypes of these species: *C. arborescens* Lam., spec. 913.5; *C. frutex* (L.) Koch, spec. 913.6; *C. pygmaea* (L.) DC., spec. 913.8. The type of the genus *Caragana* is spec. 913.5. The location of the type of *C. spinosa* (L.) DC. is unknown.

Examination of photographs[2] of the specimens of *Caragana* contained in the Linnaean Herbarium of the Linnaean Society of London has led to the conclusion that some discussion and selection of the types of the Linnaean species of this genus is justified.

In all, four species of *Caragana* were known to Linnaeus, who classed them in all his works in the genus *Robinia*. In the first edition of *Species Plantarum* (10) appear three species: *Robinia Caragana*, *R. frutex*, *R. pygmaea*. The same are found in the second edition of *Species Plantarum* (11) but here, for *frutex*, the epithet *frutescens* is used. De Candolle (2) and some other authors took up *frutescens* from the later works of Linnaeus, and the illegitimate name *C. frutescens* is still occasionally encountered. The fourth species, *R. spinosa*, was described later in the *Mantissa Plantarum altera* (12).

The genus *Caragana* was erected by Lamarck (7) who transferred thereto from *Robinia* the Linnaean species *Caragana* and *frutex* (*frutescens*). The first species, *R. caragana* L., from which the new generic name was derived, was renamed *Caragana arborescens* from the earlier phrase-name of Amman (1) cited by Linnaeus. Linnaeus' species *frutex* was renamed as *C. digitata*, an epithet descriptive of the leaf, and the Linnaean name *R. frutescens* was placed in synonymy. The former name is, of course, illegitimate and it remained for Koch to make the legitimate combination *C. frutex* (L.) Koch. Lamarck did not recognize *pygmaea* as a species but regarded it (8, p. 89) as a smaller and more spiny variant of *C. digitata*.

The Linnaean Herbarium in London contains eight sheets filed under the genus *Robinia*. Of these, five (913.5 to 913.9) are now referred to *Caragana*.

Sheet 913.5 is labelled in Linnaeus' hand "*Caragana 3*"—an obvious reference to *Robinia Caragana*, the third species of *Robinia* as treated in *Species Plantarum I*. This specimen is herewith regarded as lectotype of the species *Caragana arborescens* Lam. and as type of the genus *Caragana*. The sheet bears the Linnaean symbol usually interpreted (Stearn, 15, p. 106) as indicating that the plant was collected in western Asia, probably by Gerber in the region of the River Don or Astrachan. This specimen is representative of the species

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[2]Photographs made by the International Documentation Centre, Sweden. Specimen numbers are those of the catalogue of Savage (14).

as understood today and no difficulty presents itself in this case. The statement of Pojarkova (13) that the type of *C. arborescens* is in Paris is probably based on the assumption that material seen by Lamarck would be in that city.

Specimen 913.6 bears the words "*frutescens 4*" and the symbol indicating origin in western Asia. This Linnaean script associates it with *Robinia frutex*, the fourth species of *Robinia* in *Species Plantarum I*, for which the epithet "*frutescens*" was later used by Linnaeus. This specimen is clearly representative of the species and sheet 913.6 is chosen as lectotype of *Caragana frutex* (L.) Koch.

Sheet 913.7 bears in Linnaeus' hand "*pygmaea 5*", the symbol indicating western Asia, and, on the reverse, "*Aspalathus Amm. n. 282*". These inscriptions clearly associate the specimen with *Robinia pygmaea*, the fifth *Robinia* species in *Species Plantarum I*. The inscription on the reverse refers to the species of Amman which Linnaeus cites in synonymy of *R. pygmaea*. Unfortunately, however, although this specimen would appear to be a syntype of *C. pygmea* and it would seem preferable to designate it as lectotype, it belongs not to this species, as currently understood, but to *C. frutex*. This fact was first noted by J. E. Smith and recorded on the sheet in the words "*nil nisi R. frutescens inculta*". *Caragana frutex* is common in western Asia but *pygmaea* does not occur west of Altai. The range of *pygmaea* would doubtless be considered by Linnaeus as eastern Asia. To accept specimen 913.7 as lectotype of *C. pygmaea* (L.) DC. would greatly disturb common usage, would place the name in the synonymy of *C. frutex*, and necessitate renaming the species long and widely known as *C. pygmaea*.

The two sheets 913.8 and 913.9 are, however, representative of *C. pygmaea*, as currently used and as actually understood by Linnaeus. Neither is named in Linnaeus' hand but the former bears the symbol believed to signify that the plant was collected in eastern Asia, perhaps by Gmelin (see Stearn (15)). That specimen 913.8 is typical of *pygmaea*, as understood by 18th century authors, and conforms as well to the concept of Linnaeus is indicated by the notation of J. E. Smith, probably written about 1785, "*R. pygmaea Fl. Ross., HB. et omnium auctorum etiam Lin. Sp. Pl. I.*". To conserve common and original usage of the name *C. pygmaea*, it is necessary to reject specimen 913.7 as possible type and to select either 913.8 or 913.9. Specimen 913.7 is rejected as possible type because it does not conform to the description of *pygmaea* in *Species Plantarum I* and specimen 913.8 is here selected as lectotype of *C. pygmaea* (L.) DC.

According to Linnaeus' description, *frutex* and *pygmaea* both have 4-parted (foliate) leaves which, in *frutex*, are petiolate (*foliis quaternatis* petiolatis) but in *pygmaea* are sessile (*foliis quaternatis sessilibus*). Leaflets of *frutex* are large and broad (*major latifolius*) and of *pygmaea*, smaller and narrow (*minor angustifolius*). It seems reasonable to expect from this that leaflets of *pygmaea* would be several times as long as broad. Leaves of *frutex* are always borne on distinct petioles, approximately half or as long as the leaf, whereas leaves of *pygmaea* are sessile except on rapidly growing terminal shoots where they may be shortly petiolate.

Caragana frutex has a very extensive range and is quite variable. Individuals with smaller leaves or of low growth habit occur and are not uncommon in

cultivation. Possibly specimen 913.7, which is much smaller than the typical 913.6, was taken from such a smaller individual. In addition, it is very probable that 913.7 was taken in early spring before the leaves were fully expanded. This circumstance contributed to the small leaflet size and, more particularly, to the almost sessile condition. The latter is doubtless the feature which deceived Linnaeus in the naming of this specimen.

It is in the width of the leaflet that specimen 913.7 disagrees most markedly with the description of *pygmaea*. The larger leaflets measure 7–8 mm in length, 5 mm in width, and are obovate with a rounded apex. Such relative width to length seems too great to fit the term "narrow". Leaflets of 913.8 are linear ovate, acuminate, spiny tipped, 12 mm long, 2 mm wide, and may well be described as narrow.

It is concluded that Linnaeus, as shown by the descriptions in *Species Plantarum I*, had an accurate understanding of the two species *frutex* and *pygmaea*. He was led to label erroneously 913.7 as *pygmaea* by its apparently sessile leaves, a condition due to the unexpanded foliage of spring. To rigidly hold this sheet as the type of *C. pygmaea* would unnecessarily disturb widespread usage and destroy Linnaeus' own concept of the species. The annotations by J. E. Smith are evidence that he too believed that the determination of 913.7 as *pygmaea* was due to an oversight on the part of Linnaeus and that 913.8 truly conformed to the Linnaean concept of *pygmaea*.

The pre-Linnaean description and illustration of Amman, which Linnaeus cites in synonymy of *R. pygmaea*, and Edwards' illustration of *C. pygmaea* (3) clearly show the species typified by Linnaean specimen 913.8. The illustrated species can also be recognized as distinct from the closely related and later-described species *C. aurantiaca* Koehne by the fact that the calyx of the former is distinctly longer than broad whereas that of *C. aurantiaca* is approximately equal in length and width.

The fourth Linnaean species was described in 1771, apparently independently by Linnaeus, in *Mantissa Plantarum altera* (12) as *Robinia spinosa* and by Laxmann (9) in a thesis with an illustration, as *R. spinosissima*. It is probable that seed of this species was sent by Laxmann to Linnaeus. Laxmann states that he has distributed seed and Juel (4) records that *C. spinosa* was cultivated in the botanic garden at Uppsala in 1770. The question arises as to which specific epithet has priority. Examination of the two publications affords no proof as to which appeared the earlier in 1771. The Linnaean description includes reference to Laxmann which seems to be an acknowledgment of the source of the plants in the Uppsala garden and the information about its native range rather than a reference to the Laxmann thesis. No similarities in phraseology suggest that either author has copied from the other. Laxmann's thesis was presented on June 20, 1771, but there is no exact evidence when Volume 15 of *Novi Commentarii*, containing this and other theses, was published. All of the other theses are undated. The preface of the *Mantissa altera* is dated Sept. 1, 1771, and it appears probable that both works appeared almost simultaneously in the last quarter of the year.[3] A. P. de Candolle (2)

[3]It is stated by Stearn (p. xi, Introductory notes on Linnaeus's *Mantissa Plantarum*, prefixed to facsimile edition of Linnaeus, *Mantissa Plantarum*, 1767 and 1771, published by J. Cramer and H. K. Swann as Historiae naturalis classica VII, Weinheim, Germany, 1961) that the *Mantissa altera* was published in October 1771.

transferred this species to the genus *Caragana* as *C. spinosa*, with Laxmann's epithet in synonymy. Index Kewensis and, with one exception, subsequent authors (Komarov, Pojarkova *et al.*) have followed de Candolle, attributing priority to the Linnaean publication. However, Koch (5) took up the name *C. spinosissima*, placing the Linnaean name in synonymy, but gave no reason for this action. In the absence of definite evidence to the contrary it is necessary to follow de Candolle, who was the first author to consider the problem.

The location of the type specimen of *C. spinosa* (L.) DC. is unknown. Pojarkova (13) stated that the type was in London, apparently assuming that types of all the Linnaean species were in the Linnaean Herbarium in London. However, no specimen of this species is in the London collection. Inquiry has revealed that none is in the Linnaean collection at Stockholm (Hasselrot, private communication, 1961). Among the 70 specimens, wild and cultivated, cited as seen by Komarov (6) are none attributed to Linnaeus or Laxmann. It is hoped that this paper, by drawing attention to the lack of a type of *C. spinosa*, may yet lead to the discovery of a Linnaean specimen of this species. In the absence of a Linnaean specimen, the illustration by Laxmann testifies to the identity of the plant known, in all probability, to Linnaeus as *Robinia spinosa*. This figure is sufficiently accurate to be recognized as a member of the species *Caragana spinosa* and there seems no reason to question current usage of this name.

References

1. Amman, J. 1739. Stirpium rariorum in Imperio Rutheno . . . Icones et Descriptiones. Petropoli.
2. Candolle, A. P. de. 1825. Prodromus systematis naturalis. Vol. 2. Paris.
3. Edwards, S. 1826. The botanical register. Vol. 12, t. 1021. London. 1826.
4. Juel, H. O. 1919. Hortus Linnaeanus. Uppsala.
5. Koch, K. 1869. Dendrologie. Vol. 1. Erlangen.
6. Komarov, V. L. 1908. Prolegomena ad floras Chinae et Mongoliae. II. Generis Caraganae monographia. Acta Horti Petrop. **29**(2), 177–388.
7. Lamarck, J. B. de. 1789. Dictionnaire encyclopédique de botanique. Vol. 1. Paris.
8. Lamarck, J. B. de. 1811. Encyclopédie méthodique botanique. Suppl. 2. Paris.
9. Laxmann, E. 1771. Novae plantarum species. Novi Comment. Acad. Sci. Imp. Petropol. **15**, 558–60.
10. Linnaeus, C. 1753. Species Plantarum. Ed. I. Stockholm.
11. Linnaeus, C 1763. Species Plantarum. Ed. II. Stockholm.
12. Linnaeus, C. 1771. Mantissa Plantarum altera. Stockholm.
13. Pojarkova, A. I. 1945. *Caragana*. *In* Flora U.S.S.R. Vol. 11. Moscow and Leningrad.
14. Savage, S. 1945. A catalogue of the Linnaean Herbarium. London.
15. Stearn, W. T. 1957. An introduction to the Species Plantarum and cognate botanical works of Carl Linnaeus. (*Prefixed to* Ray Society's facsimile of Linnaeus, Species Plantarum. Vol. 1.) London.

EVOLUTION OF CARBON DIOXIDE BY TOBACCO LEAVES DURING THE DARK PERIOD FOLLOWING ILLUMINATION WITH LIGHT OF DIFFERENT INTENSITIES[1]

E. B. TREGUNNA,[2] G. KROTKOV, AND C. D. NELSON

Abstract

Detached tobacco leaves were placed in a closed-circuit apparatus and the air was continuously circulated over the leaves and through an infrared CO_2 analyzer. From the known volume of the apparatus and the percentage of carbon dioxide in its air, the amounts of carbon dioxide either absorbed or evolved by a leaf were calculated.

When, after a period of illumination, leaves were darkened, the attainment of their steady rates of respiration was preceded by two outbursts of carbon dioxide evolution. Since these outbursts occurred only after a period of illumination, it has been concluded that both were the result of photostimulation. The peak of the first outburst was usually considerably higher than that of the second. It was of short duration and the height of its peak was accentuated by the increased light intensity in the preceding light period. The second outburst lasted longer and prior light intensity had no effect on the height of its peak.

Introduction

It was assumed for a long time that respiration in light proceeds at the same rate as in darkness. Since, in light, photosynthesis and respiration occur simultaneously, and since both are measured by their O_2 or CO_2 exchange, it was difficult to verify this assumption experimentally.

Recently this assumption was tested by enriching the external medium with $(O^{18})_2$ and observing the relative rates of $(O^{18})_2$ and $(O^{16})_2$ exchange both in light and in darkness. From the earlier results obtained with this technique it was concluded that light has no effect on respiration (2, 10, 15).

Later work, however, with both O^{15} and C^{14} has shown that this may not always be the case. From the observation that the recent products of photosynthesis were transformed into the intermediates of the Krebs' cycle only in darkness, but not in light, it was suggested that light suppresses respiration (1, 5). In the photosynthetic bacterium *Rhodospirillum rubrum* oxygen consumption was decreased by light, though never suppressed completely (11). In *Anabaena* there was observed not only photoinhibition, but also photostimulation (3). From kinetic considerations it has been concluded that evolution of carbon dioxide in light is not constant. Initially, at high external concentrations of carbon dioxide, its production is greater than in darkness. The rate drops below dark respiration, as the carbon dioxide content of the air is decreased (18). In recent experiments (4), in which plants were supplied simultaneously with isotopes of both oxygen and carbon dioxide, it was shown that light has an effect on respiration. Carbon dioxide evolution was independ-

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ent of light intensity, whereas consumption of oxygen was unaffected at low light intensities and increased at high. This effect of light was more pronounced on starved cells (19).

There is, however, also a second method for studying the effect of light on respiration. If light has no effect on respiration, then immediately after illumination the rates of oxygen consumption and carbon dioxide evolution ought to be the same as those after a period of darkness. On the other hand, if light either stimulates or depresses respiration, then some transitory respiratory rates may be expected as soon as light is turned off. For *Rhodospirillum rubrum* a decreased oxygen consumption was observed immediately after a period of illumination, as compared with that after a period of darkness (11). A higher initial evolution of carbon dioxide was reported for *Chlorella* placed in darkness after a period of illumination (12).

Decker (6) placed tobacco leaves in a chamber from which air was continuously circulated through an infrared CO_2 analyzer connected to a recorder. He observed that after a period of illumination the initial rates of carbon dioxide evolution in darkness were about five times higher than those observed after 6–9 minutes. Similar results, although to a lesser extent, were observed in bean, castor bean, tulip, poplar, and white ash leaves. Photostimulated carbon dioxide production was also observed in representatives of Pteridophytes, Gymnosperms, and six species of Angiosperms (7).

In the experiments reported below the magnitude of such a photostimulation, and its relation to previous light intensity, has been investigated.

Materials and Methods

Nicotiana tabaccum, var. Connecticut Havana 38, seeds were sown in pots either in the spring or in early summer.* Plants were grown in garden soil in a greenhouse at 65–75° F under a 16-hour day, supplemented with fluorescent light. To ensure rapid growth plants were watered weekly with a solution of commercial Plant Prod 20-20-20. Leaves used for these experiments were all fully expanded. They were selected between the 10th and the 15th leaf, counting from the bottom of flowering plants.

The apparatus used to determine absorption and evolution of carbon dioxide was that described by Lister *et al.* (13). It consisted of a leaf chamber made of three $\frac{1}{4}$-in.-thick plexiglass sheets glued together. Two of these sheets were hollowed out to hold the leaf, and a fourth sheet formed the lid. A diagram of this chamber is shown in Fig. 1. A detached leaf placed in this chamber had its petiole in a small polythene bag filled with water. The volume of this chamber was 1.56 liters.

Air was continuously circulated by a pump through the chamber and an Infra-Red CO_2 Analyzer (I.R.C.A.) connected in series. The I.R.C.A. was connected to an Esterline Angus Recorder, with a chart speed of 3 in. per minute. In this manner continuous records were obtained of changes in the concentration of carbon dioxide around the leaf. Knowing the volume of the whole apparatus and the percentage of carbon dioxide in its air, it was possible

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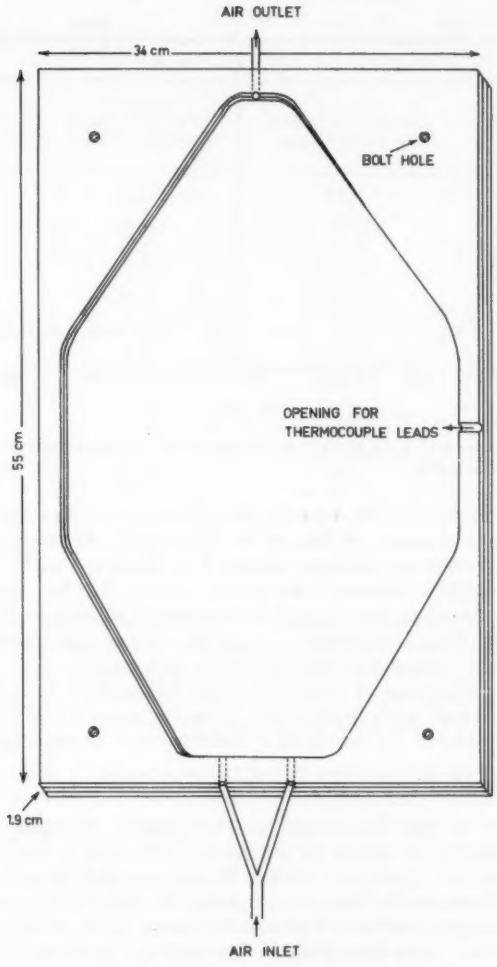

FIG. 1. Diagram of the leaf chamber.

to calculate the milligrams of carbon dioxide either added to or removed from the air. Since the total volume of the whole apparatus was 1.9 liters and the rate of circulation 2 liters/minute a complete circulation of air in the chamber occurred every minute. The time lag between the darkening of the leaf in the chamber and the change in the recorder reading was 14 seconds.

For the determination of its photosynthetic rates the leaf was illuminated with four 375-watt Sylvania photoflood lamps. The light was filtered through

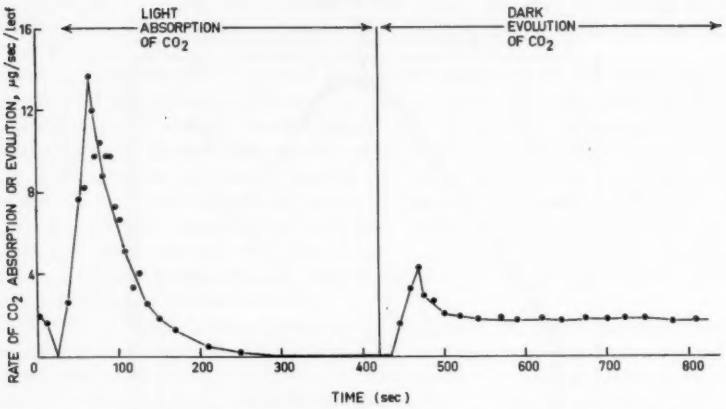

FIG. 2. Experiment 1. A typical record of carbon dioxide absorption or evolution during one of the light–dark cycles.

10 cm of water to remove the infrared portion of it, and its intensity cut down by introducing a number of sheets of Whatman chromatographic paper between the leaf chamber and the lamps. The intensity within the chamber was measured with a Weston foot-candle meter. At the edge of the leaf chamber it was found to be one-half or two-thirds of that in the middle. An average of the reading at the center and at the edge of the chamber is given in this paper as light intensity in any particular experiment.

For the determinations of respiration the lights were turned off and the chamber was covered with black cloth. The temperature of the leaf in the chamber was measured by means of a thermocouple pressed against the leaf lamina.

Experiment 1

The objective of this experiment was to observe changes in the carbon dioxide absorption or evolution by a tobacco leaf, when it was subjected to a series of consecutive light–dark cycles. To achieve this objective, a tobacco leaf of 35-g fresh weight was placed in the leaf chamber and its carbon dioxide exchange was determined for 19 successive cycles, each consisting of a light and a dark period. Light intensities in different cycles varied from 8 to 4000 ft-c and the durations of various light and dark periods between $1\frac{1}{2}$ and 45 minutes. A typical record obtained during one of the light–dark cycles is shown in Fig. 2.

At the beginning of illumination, after a brief period of carbon dioxide evolution, there was a rapid increase in the rate of carbon dioxide absorption. An average of the four highest values at the peak has been taken to represent the highest photosynthetic rate for this particular light period. Having reached this peak the rate of carbon dioxide uptake decreased, probably as a result of the depletion of the carbon dioxide of the air.

TABLE I

Experiment 1. Effects of light intensity, carbon dioxide concentration, and temperature on carbon dioxide absorption by a detached tobacco leaf

Light, ft-c	Maximal absorption rate, μg CO_2/sec/leaf	Initial CO_2 concn., μg CO_2/100 ml	Temperature change during light period, °C
8	−1.2	20.4	0
200	4.4	30.2	0
	6.3	53.1	−0.8
	6.0	104.0	+0.3
300	8.6	53.1	−0.5
	5.3	61.0	+0.8
600	4.8	36.1	+0.8
	11.5	52.1	−1.0
	9.1	122.0	+0.8
1000	4.2	24.2	+1.0
	7.9	44.8	+1.8
	—	54.0	+0.5
	12.0	65.5	−2.5
	15.0	79.4	+0.3
4000	19.3	113.0	+1.3
	10.8	42.3	+5.3
	16.0	94.1	+5.3
	12.6	110.0	+5.3

Table I shows the effects of several factors on the maximal rate of absorption of carbon dioxide observed in various light periods. From the comparison of columns 1 and 2 there appeared to be a general direct proportionality between the rate of absorption and the light intensity. There were many deviations, however, from such a relationship, suggesting that some other factors besides light were limiting.

As is seen in Fig. 2, as soon as the lights were turned off the leaf began to evolve carbon dioxide at a continuously increasing rate, which reached a peak after about 60 seconds. Then the rate of carbon dioxide evolution decreased, coming to a more or less steady value after about 100 seconds. Since these initially higher rates of carbon dioxide production occurred immediately after illumination, it is suggested that they represent the effect of photostimulation.

In some of the comparable graphs obtained for other cycles there appeared also a slight indication of a second peak, occurring about 150 seconds from the beginning of the dark period. It was thought, therefore, that photostimulation may actually produce not one, but two outbursts of carbon dioxide. The second peak, however, may not always be observed because of the following aspect of the technique used.

In this experiment air was circulating through the leaf chamber at the rate of 2 liters per minute, with only 5 per cent of it going through the I.R.C.A. The sampling tube of the I.R.C.A. has a volume of about 100 ml and the air was going through it at the rate of 50 ml per minute. This meant that it took 2 minutes to fill the I.R.C.A. sampling tube with a new air sample. Under

these conditions the second peak of carbon dioxide production could be seen only if it were considerably higher than the final dark evolution and if it were sufficiently separated in time from the first peak.

To separate these two peaks on the record chart one should bring air from the leaf chamber to the sampling tube of the I.R.C.A. as rapidly as possible. This could be done either by decreasing the volume of the whole apparatus, especially of the leaf chamber itself, or by increasing the rate of circulation through the sampling tube of the I.R.C.A., which is simpler. To observe the effects of increased circulation the following tests were carried out.

A detached tobacco leaf of 24-g fresh weight was placed in the leaf chamber and subjected to a series of consecutive light–dark cycles. In each cycle a 10-minute light period, with light intensity of 600 ft-c, was followed by a 7-minute dark period. The rate of circulation over the leaf was still 2 liters per minute, but circulation through the I.R.C.A. varied between 50 and 1450 ml per minute. As is shown in Table II, two peaks of carbon dioxide evolution were observed in all dark periods and their respective heights were recorded.

At the highest rates of air flow the difference between the two peaks became accentuated, though the height of the second peak and the final steady values were essentially constant in all periods. The first photostimulated peak was always higher than the second one. Since a further increase in the rate of circulation in the system above 250 ml per minute increased only slightly the height of the first peak, this rate was adopted for experiments 2 and 3.

Experiments 2 and 3

The magnitude of photostimulated dark evolution of carbon dioxide, as affected by the intensity of preceding illumination, was investigated further in experiments 2 and 3. Experiment 2 had 20 cycles following one another, each cycle consisting of 10 minutes of light and 7 minutes of dark. Experiment 3 had 27 of these cycles. The intensity of illumination in various light periods of both experiments varied between 200 and 4000 ft-c. In experiment 2 the fresh weight of the leaf was 25 g and in experiment 3, 32 g.

TABLE II

Effect of the rate of circulation through the I.R.C.A. on the height of the first and second peaks of photostimulated carbon dioxide evolution

Rate of flow, ml/min	Evolution of carbon dioxide, μg CO_2/sec/leaf		
	First peak	Second peak	Final rate
1450	3.8	1.8	1.5
	3.3	1.8	1.7
50	2.5	1.9	1.7
	2.5	1.9	1.7
250	3.0	2.0	1.7
	2.7	2.0	1.7
750	3.0	2.0	1.7
	3.1	2.0	1.7
1450	3.4	2.0	—
	3.2	1.9	1.6

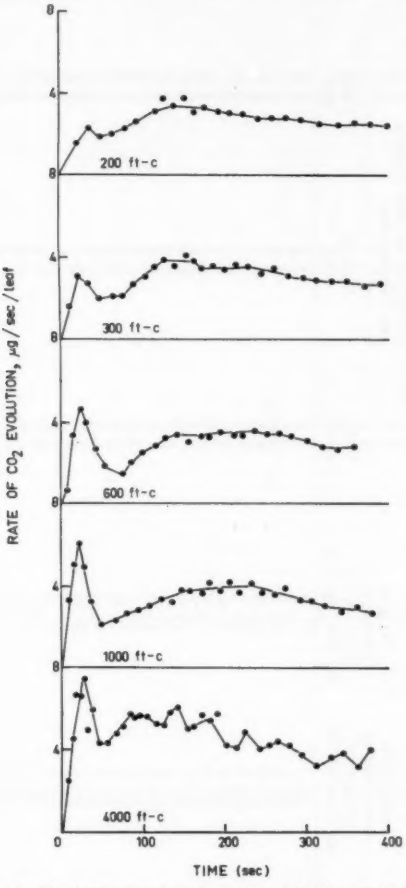

FIG. 3. Experiment 2. Typical records of carbon dioxide evolution during the dark periods, following illumination with different light intensities. Zero time on these graphs is the time when the I.R.C.A. first responded to change in carbon dioxide concentration as a result of darkening of the leaf.

Figure 3 shows a few of the typical records of carbon dioxide production obtained during the dark periods after illumination with intensities varying between 200 and 4000 ft-c. Each of these records had an initial period of rising respiration, the peak of which is designated R1. It decreased to a trough and then increased slowly again, reaching the second peak designated R2. Then carbon dioxide evolution decreased once more, reaching the lowest or the final value toward the end of the dark period.

Tables III and IV give a summary of the results obtained for various light and dark periods. Since light periods were followed immediately by the dark

TABLE III

Experiment 2. Absorption and evolution of carbon dioxide during light and dark

Cycle No.	Light intensity during the light period, ft-c	Initial CO_2 concn. during light period, μg CO_2/100 ml	Rate of absorption in light period, μg CO_2/sec/leaf	Rate of evolution in dark period, μg CO_2/sec/leaf		
				First peak	Second peak	Final rate
1	600	60.7	15.8	4.9	3.1	2.9
2	600	66.4	20.6	5.5	3.2	2.7
3	600	67.8	21.9	5.6	3.4	2.7
4	1000	70.1	25.2	7.5	3.9	2.8
5	600	79.4	25.0	5.0	3.6	—
6	600	87.2	20.6	3.9	3.5	2.7
7	600	73.1	18.5	4.0	3.8	2.9
8	4000	76.0	24.0	6.7	5.0	3.5
9	600	103.0	24.6	4.6	4.5	3.2
10	600	90.3	22.0	4.9	4.4	3.3
11	300	82.0	13.5	3.2	3.9	2.9
12	600	79.4	18.3	4.7	4.0	3.2
13	600	84.1	20.8	4.8	4.2	3.1
14	125	78.0	—*	—*	—*	—*
15	600	—*	—*	4.0	4.1	3.0
16	600	80.9	19.3	3.9	4.0	3.0
17	200	80.5	7.2	No peak (2.1)†	3.4	2.7
18	600	68.3	14.3	3.9	3.9	3.0
19	600	77.0	18.0	3.8	3.8	2.8
20	600	79.4	19.1	—	—	—

* Recorder run down. Data lost.
† Value taken from the graph at the time of the expected peak.

TABLE IV

Experiment 3. Absorption and evolution of carbon dioxide during light and dark

Cycle No.	Light intensity during the light period, ft-c	Initial CO_2 concn. during light period, μg CO_2/100 ml	Rate of absorption in light period, μg CO_2/sec/leaf	Rate of evolution in dark period, μg CO_2/sec/leaf		
				First peak	Second peak	Final rate
1	600	65.6	17.3	3.6	3.4	2.7
2	600	69.9	20.8	4.4	3.4	2.7
3	600	72.1	21.5	3.9	3.5	2.8
4	1000	72.1	23.0	5.3	4.0	3.0
5	600	84.1	21.6	3.8	3.6	2.4
6	600	82.4	20.4	4.2	3.8	2.9
7	4000	78.0	29.7	6.5	5.6	3.8
8	600	108.0	28.0	5.2	4.6	3.0
9	600	111.0	28.0	5.0	4.4	3.2
10	600	87.2	26.0	4.8	4.3	3.1
11	300	88.7	15.0	2.6	3.8	2.5
12	600	93.1	23.2	4.9	4.1	2.7
13	600	103.6	26.0	4.4	4.2	3.1
14	600	82.4	24.2	4.6	4.1	2.9
15	200	93.5	8.8	No peak (2.1)*	3.6	2.5
16	600	70.6	17.3	4.4	4.1	3.1
17	600	88.7	26.0	4.4	4.2	3.0
18	200	85.7	8.0	No peak (2.1)*	3.7	2.7
19	600	69.1	—	4.0	4.0	2.8
20	600	80.9	17.8	3.5	3.8	3.0
21	300	87.2	10.8	2.2	3.5	2.7
22	600	68.3	15.0	3.5	4.2	2.9
23	600	80.0	18.0	4.2	4.0	3.2
24	1000	79.4	20.8	4.6	4.4	4.0
25	600	72.1	17.9	3.5	4.0	2.8
26	600	76.5	18.9	3.2	3.8	3.0
27	600	76.5	18.3	3.1	4.0	3.0

* Value taken from the graph at the time of the expected peak.

ones, the initial concentration of carbon dioxide at the beginning of illumination was also the same as that reached at the end of the preceding dark period.

From the examination of Tables III and IV it is seen that the final rate of production of carbon dioxide, reached at the end of each dark period, was practically always preceded by two peaks of higher rates. The first peak was usually higher than the second one and its magnitude was affected directly by the light intensity in the preceding light period.

Discussion

It is clear from the data obtained in experiments 2 and 3 that tobacco leaves placed in darkness after a period of illumination did not settle at once to a steady rate of carbon dioxide production, but first had two outbursts. The first outburst, as is seen from Fig. 3, was short though the rate of carbon dioxide evolution at its peak was high. The second outburst lasted longer, but the rate at its peak was much lower. Since both outbursts occurred immediately after a period of illumination, one may assume that both were photostimulated.

The height of the first outburst was markedly and directly proportional to the intensity of illumination during the preceding light period. The height of the second outburst, however, was essentially the same no matter whether the illumination was 200 or 1000 ft-c. Somewhat higher values obtained at 4000 ft-c might be due to a temperature rise at this light intensity.

To examine further the relationship between the magnitude of the first photostimulated outburst and the preceding light intensity the data presented in Tables III and IV were recalculated in the following manner.

From the second column of each table pairs of adjacent cycles were selected, in which the first light period had a light intensity of 600 ft-c and the second one either higher or lower intensity. The heights of the first peaks of carbon dioxide evolution in the two corresponding dark periods were compared. The difference between these two peaks was taken as a measure of the photostimulation brought about by the difference in the light intensities between their respective light periods. The difference between the heights of the two peaks divided by the difference in the light intensities gave a value for the photostimulation per unit of illumination. Table V presents the results of such calculations.

Two main conclusions can be drawn from this table. First, after light intensity of either 200 or 300 ft-c the magnitude of photostimulation per 100 ft-c was high, being 0.55 and 0.56 μg CO_2/sec/leaf, respectively. Photostimulation decreased to 0.31 at 1000 ft-c and further to 0.08 at 4000 ft-c. In other words at the low intensity of 200–300 ft-c there is a linear relationship between the light intensity and photostimulation. The amount of photostimulation per 100 ft-c begins to decrease between 600 and 1000 ft-c and is very low at 4000 ft-c. Second, the residual carbon dioxide evolution is low at low light intensity, but it begins to increase rapidly at light intensities somewhere between 300 and 1000 ft-c.

The existence of two outbursts of photostimulated carbon dioxide evolution indicates at least two different effects of light. The first outburst comes suddenly, it rapidly reaches its peak, and decreases equally rapidly. Since its peak is high and narrow, the magnitude of this outburst may be conveniently

TABLE V

Experiments 2 and 3. Effect of preceding light intensity on the magnitude of the first photostimulated carbon dioxide outburst in the following dark period

Experiment and cycle nos.	First peak, as μg CO_2/sec/leaf after different light intensities		Difference	Photostimulation per 100 ft-c, μg CO_2/sec/leaf*	Total photostimulation, μg CO_2/sec/leaf at different light intensities		Residual carbon dioxide evolution,† μg CO_2/sec/leaf
	600 ft-c	*200 ft-c*			*600 ft-c*	*200 ft-c*	
Expt. 2, cycles 16–17	3.9	2.1‡	1.8	0.45	2.70	0.90	1.2
3 14–15	4.6	2.1‡	2.5	0.63	3.78	1.26	0.8
3 17–18	4.4	2.1‡	2.3	0.58	3.48	1.16	0.9
				0.55	3.30	1.10	1.0
	600 ft-c	*300 ft-c*			*600 ft-c*	*300 ft-c*	
Expt. 2, cycles 10–11	4.9	3.2	1.7	0.57	3.42	1.71	1.5
3 10–11	4.8	2.6	2.2	0.73	4.38	2.19	0.4
3 20–21	3.5	2.2	1.3	0.43	2.58	1.29	0.9
				0.56	3.36	1.68	0.9
	600 ft-c	*1000 ft-c*			*600 ft-c*	*1000 ft-c*	
Expt. 2, cycles 3–4	5.6	7.5	1.9	0.48	2.88	4.80	2.7
3 3–4	3.9	5.3	1.4	0.35	2.10	3.50	1.8
3 23–24	4.2	4.6	0.4	0.10	0.60	1.00	3.6
				0.31	1.86	3.10	2.7
	600 ft-c	*4000 ft-c*			*600 ft-c*	*4000 ft-c*	
Expt. 2, cycles 7–8	4.0	6.7	2.7	0.08	0.48	3.20	3.5
3 6–7	4.2	6.5	2.3	0.07	0.42	2.80	3.7

* These values have been calculated on the assumption that the height of the first peak does represent the true magnitude of photostimulation. If the photostimulation is determined in some different way, e.g. by extrapolating the decreasing arm of the first peak to zero time, then the absolute values given in this column would be changed accordingly.

† Carbon dioxide evolution at the peak minus total photostimulated respiration.

‡ Since there was no peak at 200 ft-c, the value for the actual respiration at this time is taken.

measured by the height of the peak. The magnitude of this outburst is increased by the intensity of illumination. No direct relation has been found between the magnitude of this outburst and the photosynthetic rate in the preceding light period, as was observed by Decker (9). This first outburst may represent the magnitude of photostimulated respiration (8) or at least a part of it may be due to photooxidation (14, 17). An increased oxygen consumption under the effect of blue light has been reported for etiolated corn leaves and for the yellow portions of variegated leaves of several plant species (16).

On the other hand, though the height of the second peak is lower than that of the first, the second outburst of carbon dioxide is extended for a considerably longer period of time and it may begin much earlier than its observed peak.

From the very fact that there are two photostimulated outbursts of carbon dioxide production, differing in their timing, magnitudes, and dependence on previous light intensity, one can infer that light affects evolution of carbon dioxide in more than one way. For the further elucidation of these two effects more work is necessary.

References

1. Benson, A. A. and Calvin, M. 1950. The path of carbon in photosynthesis. VII. Respiration and photosynthesis. J. Exptl. Botany, **1**, 63–68.
2. Brown, A. H. 1953. The effect of light on respiration using isotopically enriched oxygen. Am. J. Botany, **40**, 719–729.
3. Brown, A. H. and Webster, G. C. 1953. The influence of light on the rate of respiration of the blue-green alga, *Anabaena*. Am. J. Botany, **40**, 753–758.
4. Brown, A. H. and Weis, D. 1959. Relation between respiration and photosynthesis in the green alga, *Ankistrodesmus braunii*. Plant Physiol. **34**(3), 224–234.
5. Calvin, M. and Massini, P. 1952. The path of carbon in photosynthesis. XX. The steady state. Experientia, **8**, 445–484.
6. Decker, J. P. 1955. A rapid, post-illumination deceleration of respiration in green leaves. Plant Physiol. **30**(1), 82–84.
7. Decker, J. P. 1957. Further evidence of increased carbon dioxide production accompanying photosynthesis. J. Solar Energy Sci. Eng. **1**, 30–33.
8. Decker, J. P. and Wien, J. D. 1958. Carbon dioxide surges in green plants. J. Solar Energy Sci. Eng. **2**(1), 39–41.
9. Decker, J. P. 1959. Comparative responses of carbon dioxide outburst and uptake in tobacco. Plant Physiol. **34**(2), 100–102.
10. Gaffron, H. and Fager, E. W. 1951. The kinetics and chemistry of photosynthesis. Ann. Rev. Plant Physiol. **2**, 87–114.
11. Johnston, J. A. and Brown, A. H. 1954. The effect of light on the oxygen metabolism of the photosynthetic bacterium, *Rhodospirillum rubrum*. Plant Physiol. **29**(2), 177–182.
12. Kok, B. and Spruitt, C. J. P. 1956. High initial rates of gas-exchange in respiration and photosynthesis of *Chlorella*. Biochem. Biophys. Acta, **19**, 212–223.
13. Lister, G. R., Krotkov, G., and Nelson, C. D. 1961. A closed-circuit apparatus with an infrared CO_2 analyzer and a Geiger tube for continuous measurement of CO_2 exchange in photosynthesis and respiration. Can. J. Botany, **39**, 581–591.
14. Rabinowitch, E. I. 1945. Photosynthesis and related processes. Interscience Publishers, Inc. Vol. I. pp. 561–571. *Also* Vol. II, Part 2. pp. 1925–1939. 1956.
15. Van Norman, R. W. and Brown, A. H. 1952. The relative rates of photosynthetic assimilation of isotopic forms of carbon dioxide. Plant Physiol. **27**(4), 691–709.
16. Voskresenskaia, N. P. and Zak, E. G. 1957. O pogloshchenii kisloroda list'iami rastenii v razlichnykh uchastkakh spektra (Absorption of oxygen by plants in different parts of a spectrum). Doklady Akad. Nauk S.S.S.R. **114**, 375–378.
17. Voskresenskaia, N. P. and Grishina, G. S. 1960. Pogloshchenie kisloroda zelenymi list'iami v zavisimosti ot intensivnosti i spektral'nogo sostava sveta (Effect of intensity and spectral composition of light on oxygen absorption by green plants). Fiziol. Rastenii Akad. Nauk S.S.S.R. **7**(5), 498–507.
18. Weigl, J. W., Warrington, P. M., and Calvin, M. 1951. The relation of photosynthesis to respiration. J. Am. Chem. Soc. **73**, 5058–5063.
19. Weis, D. and Brown, A. H. 1959. Kinetic relationship between photosynthesis and respiration in the algal flagellate, *Ochromonas malhemensis*. Plant Physiol. **34**(3), 235–239.

CHROMOSOME NUMBERS OF CANADIAN WEEDS. III[1]

GERALD A. MULLIGAN

Abstract

The chromosome numbers determined from material of 55 Canadian weeds collected in the wild are reported. The chromosome numbers for 45 of the 55 weeds studied were previously obtained by other workers, mostly on non-Canadian material. The chromosome numbers of the following 10 taxa are reported for the first time: *Chenopodium berlandieri* Moq. var. *zschackei* (Murr.) Murr. $2n = 36$; *Chenopodium botrys* L. $2n = 18$; *Corispermum villosum* Rydb. $2n = 18$; *Iva axillaris* Pursh $2n = 36$ and 54; *Lactuca pulchella* (Pursh) DC. $2n = 18$; *Descurainia pinnata* (Walt.) .Britt. ssp. *brachycarpa* (Richards.) Detling $2n = 28$; *Descurainia richardsonii* (Sweet) O. E. Schulz ssp. *richardsonii* $2n = 14$; *Descurainia sophioides* (Fisch.) O. E. Schulz $2n = 14$; *Dracocephalum thymiflorum* L. $2n = 20$; *Sium suave* Walt. $2n = 22$.

Introduction

Mulligan (25, 26) reported the chromosome numbers of 96 weeds, all determined from material growing wild in Canada. In the present paper, the third of this series, the chromosome numbers of another 55 weeds are listed, and the significance of some of the chromosome number determinations is discussed. Many of the chromosome numbers included in the papers of this series were utilized in a study of polyploidy in weeds (Mulligan (27)).

Materials and Methods

Most of the chromosome number determinations were made on greenhouse plants grown from seed collected in the wild. In a few instances, the plants studied were transferred from field infestations into the greenhouse or root tips were taken directly from plants growing in their natural habitats. Root tips were taken from each plant, placed in water, and then exposed to a temperature of 2–4° C for 2 to 3 hours. They were then fixed in Randolph's modification of Nawaschin fixative, embedded in paraffin, sectioned, and stained in crystal violet. Specimens of plants from which chromosome number determinations were made were collected and placed in the herbarium of the Plant Research Institute, Canada Department of Agriculture, Ottawa (DAO). The drawings were made with the aid of a camera lucida.

Results

A complete list of the weeds studied, their chromosome numbers, and the chromosome number determinations obtained by previous workers are given in Table I. The author's collection number appears, in parentheses, after the name or initials of the worker who collected the material. In some cases the plants studied died before voucher specimens were collected. Where these plants had been grown from seed off herbarium specimens, the collection number of the collector of the herbarium specimen is included, unbracketed, after his name or initials.

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TABLE I

Chromosome numbers of 55 Canadian weeds and references to previous counts

Species counted	Author's count, $2n$	Source of material, collector, and collection No.	Previous counts, $2n$	References to previous counts
Amaranthaceae				
Amaranthus				
powellii S. Wats.	34	Creston, B.C., G.A.M. (2159)	34	(9)—Can., U.S.A.
retroflexus L.	34	Simpson, Sask., C. Frankton (2556)	34	(9) and others—Can., U.S.A., Eur.
Asclepiadaceae				
Asclepias				
syriaca L.	22	Ottawa, G.A.M. (no voucher specimen)	22	(23) and others—Can., Eur.
Caryophyllaceae				
Stellaria				
media (L.) Cyrillo	40	Ottawa, G.A.M. (2110)	28	(29)—Asia
			40	(2) and others—Eur.
			42	(30)—Eur.
			44	(2) and others—Eur.
Chenopodiaceae				
Chenopodium				
berlandieri Moq. var. *zschackei* (Murr.) Murr.	36	Moose Jaw, Sask., D. R. Lindsay (2112)	—	
botrys L.	18	Smith Falls, Ont., W. Shumovich 1460	16	(15)—Asia
Corispermum				
hyssopifolium L.	18	Battle River, Alta., E. H. Moss (2113)	18	(31)—Eur.
villosum Rydb.	18	Greenstreet, Sask., J. L. Patmore (2114)	—	
Salsola				
kali L. var. *tenuifolia* Tausch	36	Battleford, Sask., I. J. Bassett (2451)	36	(13) and others—U.S.A., Eur.
Compositae				
Arctium				
minus (Hill) Bernh.	36	Ironsides, P.Q., G.A.M. (2389)	32	(43)—Eur.
			36	(38)—Eur.
tomentosum Mill.	36	Chatham, N.B., G.A.M. (2528)	36	(38)—Eur.
Erigeron				
strigosus Muhl.	27	Monckland Station, Ont., G.A.M. (2122)	27	(22) and others—Can., U.S.A., Eur.
			36	(22)—Can.
Hieracium				
florentinum All.	36	Ottawa, G.A.M. (1484)		
	45	Iron Bridge, Ont., I.J.B. (1533)	45	(8)—Eur.

Iva				
axillaris Pursh	36	Rounthwaite, Man., I.J.B. (2561); Success, Sask., I.J.B. (2562); Saskatoon, Sask., G. W. Selleck (2563); Laura, Sask., I.J.B. (2564); Patricia, Alta., I.J.B. (2565); Idaho Falls, Idaho, H. A. Senn (2566); Thistle, Utah, A. R. Kruckeberg (2567)	—	
	54	6 mi. N. Brandon, Man., G. Stevenson (2568)	—	
Lactuca				
pulchella (Pursh) DC.	18	Napinka, Man., I.J.B. (2459)	—	
Senecio				
vulgaris L.	40	Chilliwack, B.C., D. Faris (1501)	40	(18) and others—Eur.
Cruciferae				
Descurainia				
pinnata (Walt.) Britt. ssp. *brachycarpa* (Richards.) Detling	28	Carman, Alta., D.R.L. (2126)	—	
ssp. *filipes* (Gray) Detling	14	Mt. Greer, Vanderhoof, B.C., J. A. Calder 14490	14	(1)—U.S.A.
richardsonii (Sweet) O. E. Schulz ssp. *richardsonii*	14	Baie St. Paul, P.Q., E. G. Anderson 1220; MacRae, Yukon Territory, J. M. Gillett 4497	—	
sophia (L.) Webb.	28	Stirling, Alta., D.R.L. (2127)	28	(18) and others—U.S.A., Eur.
sophioides (Fisch.) O. E. Schulz	14	Mayo, Y.T., J.A.C. 4216	—	
Lepidium				
bourgeauanum Thell.	32	St. Simeon, P.Q., I.J.B. (2423); Alexander Falls, Mack. Dist., N.W.T., W. H. Lewis (2418); Norman Wells, Mack. Dist., N.W.T., W. J. Cody (2419)	32	(28)—Can.
densiflorum Schrad. var. *densiflorum*	32	French River, Ont., K. K. Bragg (2413)	32	(28) and others—Can., U.S.A., Eur.
var. *elongatum* (Rydb.) Thell.	32	Ashnola River, Flatiron Mountain, B.C., I. M. Cowan (2422)	32	(28)—Can.
var. *pubicarpum* (Nelson) Thell.	32	Osoyoos, B. C., G.A.M. (2412)	32	(28)—Can.
ramosissimum A. Nels.	64	Yellowknife, N.W.T., W.J.C. (2417); Edmonton, Alta., C.F. (2424)	64	(28)—Can.
virginicum L.	32	St. Thomas, Ont., L. E. James (2420); Saanichton Spit, B.C., W. Newton (2421)	32	(28) and others—Can., U.S.A., Eur.
Raphanus				
raphanistrum L.	18	10 mi. S. W. Fredericton, N.B., G.A.M. (2555)	18	(18) and others—Eur.
Sisymbrium				
altissimum L.	14	Ottawa, C.F. (1471)	14	(18) and others—U.S.A., Eur.
Euphorbiaceae				
Euphorbia				
esula L.	60	Wynyard, Sask., G. W. Selleck (2139)	60	(24) and others—Can., Eur.

TABLE I (*Concluded*)

Chromosome numbers of 55 Canadian weeds and references to previous counts

Species counted	Author's count, 2n	Source of material, collector, and collection No.	Previous counts, 2n	References to previous counts
Gramineae				
Echinochloa crusgalli (L.) Beauv.	54	Wrigley Corners, Ont., W. G. Dore (2553); Ottawa, W. G. D. (2554)	36	(3) and others—U.S.A., Eur.
			42	(4)—U.S.A.
			54	(39) and others—Eur., Asia
Labiatae				
Dracocephalum thymiflorum L.	20	Berwick, Ont., G.A.M. (2518)	—	
Glechoma hederacea L.	36	Kensington, P.E.I., H. J. Clark (2569); Bryson, P.Q., J. Ostrom (2570); Ottawa, C.F. (2571); Conc. 3, Reach Twp., Ont. Co., I.J.B. (2572)	18	(36) and others—Eur., Asia
			24	(17)—Eur.
			36	(32) and others—Eur.
Leonurus cardiaca L.	18	Burritts Rapids, Ont., G.A.M. (2453)	18	(32) and others—Eur.
Leguminosae				
Vicia angustifolia (L.) Reichard	12	Sandringham, Ont., G.A.M. (1522)	12	(42) and others—Eur.
cracca L.	28	Ironsides, P.Q., G.A.M. (2529)	12	(37) and others—Eur., Asia
			14	(37) and others—U.S.A., Eur., Asia
			28	(37) and others—Eur., Asia
Malvaceae				
Malva neglecta Wallr.	42	Collingwood, Ont., I.J.B. (3210)	42	(34) and others—Eur.
Onagraceae				
Epilobium glandulosum Lehm. var. *adenocaulon* (Haussk.) Fern.	36	Ottawa, G.A.M. (2462)	36	(16)—U.S.A.
Plantaginaceae				
Plantago lanceolata L.	12	Sicamous, B.C., J.A.C. (2465)	12	(20) and others—U.S.A., Eur., Asia
			24	(20)—Eur.
			96	(20)—Eur.

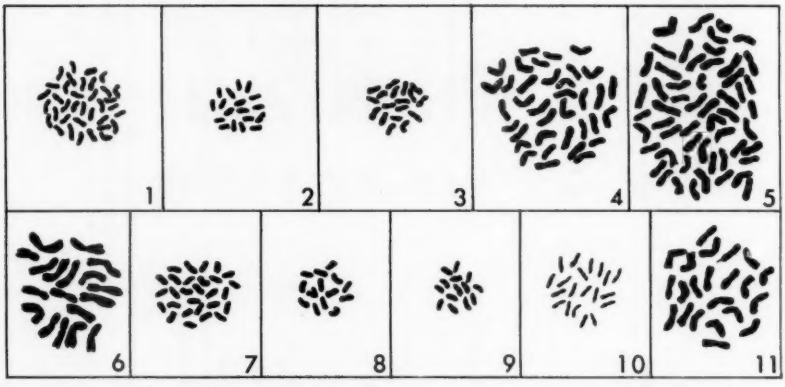
Polygonaceae				
Polygonum				
convolvulus L.	40	Round Table, Ont., C. E. Garton (2291); Galt., Ont., C.F. (2292); Makaroff, Man., C.F. (2293)	20 40	(14)—Eur. (25) and others—Can., Eur.
scabrum Moench.	22	Charlottetown, P.E.I., E.G.A. (1437); Kentville, N.S., E.G.A. (1438)	22	(14) and others—Eur.
Rumex				
thyrsiflorus Fingerh.	14, 15	Dalmeny, Sask., I.J.B. (2450)	14, 15	(19) and others—Can., Eur.
venosus Pursh	40	Burdette, Alta., J. J. Sexsmith (n.v.s.)	40	(33)—Can.
Portulacaceae				
Portulaca				
oleracea L.	ca. 54	Ottawa, G.A.M. (1520)	18 54	(10)—Asia (10) and others—Eur., Asia
Rhamnaceae				
Rhamnus				
cathartica L.	24	Ottawa, G.A.M. (1475)	24	(44)—Eur.
Scrophulariaceae				
Linaria				
vulgaris Mill.	12	Ottawa, G.A.M. (n.v.s.)	12	(41) and others—Eur.
Verbascum				
thapsus L.	36	Ottawa, G.A.M. (n.v.s.)	34, 36	(11)—Eur.
Solanaceae				
Solanum				
americanum Mill.	24	Iroquois, Ont., W.G.D. (2526); St. Thomas, Ont., Ont., L. E. James (2520)	24	(35)—U.S.A.
nigrum L.	72	Cape Forchu, N.S., E. C. Smith (2519)	72	(35) and others—U.S.A., Eur., Asia, New Zealand
nodiflorum Jacq.	24	Rosedale, B.C., H.A.S. (2523); Davis, Calif., J. McCaskell (2525)	24	(35) and others—U.S.A., N.Z.
sarachoides Sendt.	24	Salt Springs Island, B.C., W.E. Molyneaux (2521); Penticton, B.C., G.A.M. (2524)	24	(35) and others—U.S.A., S. Amer.
Umbelliferae				
Anthriscus				
sylvestris (L.) Hoffm.	16	Ottawa, G.A.M. (2454)	16	(18) and others—Eur.
Daucus				
carota L.	18	Ottawa, G.A.M. (2560)	18	(12) and others—U.S.A., S. Amer., Eur.
Sium				
suave Walt.	22	Ottawa, G.A.M. (2463)	—	
Verbenaceae				
Verbena				
hastata L.	14	Ottawa, G.A.M. (2464)	14	(6)—Eur.

Discussion

Chromosome numbers for 10 of the 55 weeds studied have not previously been reported: *Chenopodium berlandieri* var. *zschackei* $2n = 36$ (Fig. 1); *Chenopodium botrys* $2n = 18$ (Fig. 2); *Corispermum villosum* $2n = 18$ (Fig. 3); *Iva axillaris* $2n = 36$ (Fig. 4) and $2n = 54$ (Fig. 5); *Lactuca pulchella* $2n = 18$ (Fig. 6); *Descurainia pinnata* ssp. *brachycarpa* $2n = 28$ (Fig. 7); *Descurainia richardsonii* ssp. *richardsonii* $2n = 14$ (Fig. 8); *Descurainia sophioides* $2n = 14$ (Fig. 9); *Dracocephalum thymiflorum* $2n = 20$ (Fig. 10); *Sium suave* $2n = 22$ (Fig. 11).

Chenopodium berlandieri var. *zschackei*, $2n = 36$, and *C. botrys*, $2n = 18$, have a basic chromosome number of $x = 9$. Kawatani and Ohno (15) reported $n = 8$ for *C. botrys*. They also published chromosome numbers for several other species of *Chenopodium*, all having a basic number of $x = 8$. Chromosome numbers reported for this genus by all other workers have the basic number $x = 9$. The conclusion must be drawn that the chromosome number determinations for *Chenopodium* by Kawatani and Ohno are probably incorrect.

Plants of *Iva axillaris* collected from seven different locations in Canada and the United States were found to have 36 somatic chromosomes. However, one clone of this species collected 6 miles north of Brandon, Manitoba, had a higher chromosome number, $2n = 54$. No morphological characters were found that would readily differentiate between plants at the two chromosome levels. Unpublished information by I. J. Bassett of the Plant Research Institute, Ottawa, has revealed that the $2n = 54$ plants have pollen grains measurably larger than those of the $2n = 36$ plants. Other chromosome



FIGS. 1–11. Somatic chromosome complements, ×1550. Fig. 1. *Chenopodium berlandieri* var. *zschackei*, $2n = 36$. Fig. 2. *Chenopodium botrys*, $2n = 18$. Fig. 3. *Corispermum villosum*, $2n = 18$. Fig. 4. *Iva axillaris*, $2n = 36$. Fig. 5. *Iva axillaris*, $2n = 54$. Fig. 6. *Lactuca pulchella*, $2n = 18$. Fig. 7. *Descurainia pinnata* ssp. *brachycarpa*, $2n = 28$. Fig. 8. *Descurainia richardsonii* ssp. *richardsonii*, $2n = 14$. Fig. 9. *Descurainia sophioides*, $2n = 14$. Fig. 10. *Dracocephalum thymiflorum*, $2n = 20$. Fig. 11. *Sium suave*, $2n = 22$.

numbers known in the genus *Iva* are for *Iva xanthifolia* Nutt., $2n = 36$, and *Iva ciliata* Willd., $2n = 34$. Therefore, the plants of *Iva axillaris* with 36 somatic chromosomes might be diploid or tetraploid and the $2n = 54$ plant a triploid or a hexaploid. Pollen grains from the $2n = 54$ plant were stained with cotton blue in lactophenol. The pollen grains staining a dark blue color were considered viable. Of 100 pollen grains examined, 92 were classed as viable. Pollen mother cells from the $2n = 54$ plant were also studied and 27 bivalents were observed at metaphase I. Meiosis was also regular during all stages from anaphase I to telophase II. The regular meiosis and high percentage of viable pollen grains in the $2n = 54$ plant of *Iva axillaris* suggest that this plant is not a triploid but more likely a hexaploid. The $2n = 36$ plants would therefore be tetraploid.

Lactuca pulchella, $2n = 18$, a species native to North America, has the same basic chromosome number as many Old World species of *Lactuca*. Other North American species of *Lactuca*, so far studied, have a basic number of $x = 17$.

Descurainia pinnata ssp. *brachycarpa*, $2n = 28$, *D. richardsonii* ssp. *richardsonii*, $2n = 14$, and *D. sophioides*, $2n = 14$, all have the basic chromosome number $x = 7$, the same as that of *Descurainia* species studied by other workers. Baldwin and Campbell (1) listed diploid, tetraploid, and hexaploid chromosome numbers for both *D. pinnata* and *D. richardsonii* and correlated the chromosome numbers with the subspecies that were previously recognized by Detling (7). His subspecies of *D. pinnata* and *D. richardsonii* are now known to have the following chromosome numbers: *D. pinnata* sspp. *filipes* and *nelsonii* $2n = 14$, sspp. *brachycarpa*, *glabra*, and *intermedia* $2n = 28$, and ssp. *halictorum* $2n = 28$ (glandular plants) and $2n = 42$ (nonglandular plants); *D. richardsonii* sspp. *richardsonii* and *viscosa* $2n = 14$, ssp. *procera* $2n = 28$, and ssp. *incisa* $2n = 42$.

Chromosome numbers for 45 of the taxa studied in this paper have previously been reported by other workers. Thirty-four of these taxa have only one chromosome number and the same number was also obtained on Canadian material. Two or more chromosome numbers have been reported for the other 12 taxa. These 12 taxa are discussed below.

European and Asiatic workers have shown that *Stellaria media* has the chromosome numbers $2n = 28$, 40, 42, and 44. Plants from one location in Canada had 40 somatic chromosomes. Further chromosome number determinations may reveal that plants with other chromosome numbers also occur in Canada.

Wulff (43) and Tarnavaschi (38) have reported the chromosome numbers $2n = 32$ and 36, respectively, for European material of *Arctium minus*. The $2n = 36$ chromosome number was obtained on material of this species from one location in Canada. Chromosomes in the metaphase plates studied were poorly stained and clumped together. Many slides were examined and several adjustments were made in fixation and staining before a satisfactory chromosome number determination was obtained for *A. minus*. The $2n = 32$ chromosome number reported by Wulff (43) may be erroneous because of difficulties in obtaining preparations of *A. minus* chromosomes suitable for study.

Our native apomict, *Erigeron strigosus*, has the chromosome numbers $2n = 27$ and 36. In Canada, plants with the 27 chromosome number appear to be commoner than plants with the 36 number (Montgomery and Yang (22)).

Hieracium florentinum is an introduced apomict with the chromosome numbers $2n = 36$ and 45. Plants with both of these chromosome numbers occur in Canada.

The $2n = 28$ chromosome number obtained on Canadian material of *Descurainia sophia* has been confirmed by other workers on material from Europe and the United States. An octoploid chromosome number, credited to Manton (21), appears in all chromosome lists. However, her paper stated that only a few octoploid cells occurred in normally tetraploid plants.

Previous workers have reported the chromosome numbers $2n = 36$, 42, and 54 for plants of *Echinochloa crusgalli* from Europe, Asia, and the United States. The chromosome number obtained from material collected at two locations in Canada was $2n = 54$. Since plants with the numbers $2n = 36$ and 42 were found in the United States, plants with one or both of these numbers may also occur here.

The chromosome numbers $2n = 18$, 24, and 36 have been reported for European and Asiatic material of *Glechoma hederacea*. Material obtained from four locations in Canada had the chromosome number $2n = 36$. The presence of three chromosome races in this species needs clarification because of the frequent confusion of *Glechoma hederacea* with some of its near relatives.

Plants of *Vicia cracca* with the chromosome numbers $2n = 12$, 14, and 28 occur in Europe, Asia, and the United States. Material collected from one location in Canada had the chromosome number $2n = 28$.

The chromosome numbers $2n = 12$, 24, and 96 have been reported for European material of *Plantago lanceolata*. Most of the European plants studied had the diploid chromosome number. All material of *P. lanceolata* studied from Canada and the United States has also been diploid.

European workers have reported a chromosome number of $2n = 40$ for *Polygonum convolvulus*, the same number that was obtained on material of this species from four locations in Canada. The $2n = 20$ chromosome number reported for *P. convolvulus* by Jaretzky (14) was almost certainly obtained from material of another species of this genus.

The chromosome numbers $2n = 18$ and 54 have been reported for material of *Portulaca oleracea* from Asia but only the hexaploid race is known in Europe. The $2n = 54$ chromosome number was also determined from material collected at one location in Canada.

Håkansson (11) reported the chromosome numbers $2n = 34$ and 36 for European material of *Verbascum thapsus*. The Canadian material studied had the latter chromosome number.

References

1. Baldwin, J. T. and Campbell, J. M. 1940. Chromosomes of Cruciferae I. *Descurainia*. Am. J. Botany, **27**, 915–917.
2. Blackburn, K. B. and Morton, J. K. 1957. The incidence of polyploidy in the Caryophyllaceae of Britain and Portugal. New Phytologist, **56**, 344–351.
3. Brown, W. V. 1948. A cytological study in the Gramineae. Am. J. Botany, **35**, 382–395.
4. Church, G. L. 1929. Meiotic phenomena in certain Gramineae. Botan. Gaz. **88**, 63–84.

5. DARLINGTON, C. D. and WYLIE, A. P. 1955. Chromosome atlas of flowering plants. George Allen and Unwin Ltd., London.
6. DERMEN, H. 1936. Cytological study and hybridization in two sections of *Verbena*. Cytologia, **7**, 160–175.
7. DETLING, L. E. 1939. A revision of the North American species of *Descurainia*. Am. Midland Naturalist, **22**, 481–520.
8. GENTSCHEFF, G. *In* G. Tischler, Ref. 40.
9. GRANT, W. F. 1959. Cytogenetic studies in *Amaranthus*. III. Chromosome numbers and phylogenetic aspects. Can. J. Genet. Cytol. **1**, 313–328.
10. HAGERUP, O. 1932. Über Polyploidie in Beziehung zu Klima, Ökologie und Phylogenie. Chromosomenzahlen aus Timbuktu. Hereditas, **16**, 19–40.
11. HÅKANSSON, A. *In* C. D. Darlington and A. P. Wylie, Ref. 5.
12. HÅKANSSON, A. 1953. Some chromosome numbers in Umbelliferae. Botan. Notiser, **3**, 301–307.
13. HEISER, C. B. and WHITAKER, T. W. 1948. Chromosome number, polyploidy, and growth habit in California weeds. Am. J. Botany, **35**, 179–186.
14. JARETZKY, R. 1928. Histologische und karyologische Studien an Polygonaceen. Jahrb. wiss. Botan. **69**, 357–490.
15. KAWATANI, T. and OHNO, T. 1950. Chromosome numbers of genus *Chenopodium*, I. Chromosome numbers of Mexican tea (*Chenopodium ambrosiodes* L.), American wormseed (*Chenopodium ambrosiodes* L. var. *anthelminticum* A. Gray) and some allied species. Japan. J. Genet. **25**, 177–180.
16. LEWIS, H., RAVEN, P. H., VENKATESH, C. S., and WEDBERG, H. L. 1958. Observations of meiotic chromosomes in the Onagraceae. Aliso, **4**, 73–86.
17. Löve, A. and Löve, D. 1948. Chromosome numbers of northern plant species. Reykjavik, 1–131.
18. Löve, A. and Löve, D. 1956. Cytotaxonomical conspectus of the Icelandic flora. Acta Horti Gotoburgensis, **20**, 65–290.
19. Löve, A. and Löve, D. 1957. *Rumex thyrsiflorus* new to North America. Rhodora, **59**, 1–5.
20. McCALLAGH, D. 1934. Chromosomes and chromosome morphology in Plantaginaceae I. Genetica, **16**, 1–7.
21. MANTON, I. 1932. Introduction to the general cytology of the Cruciferae. Ann. Botany, **46**, 509–556.
22. MONTGOMERY, F. H. and YANG, S.-J. 1960. Cytological studies in the genus *Erigeron*. Can. J. Botany, **38**, 381–386.
23. MOORE, R. J. 1946. Investigations on rubber-bearing plants. IV. Cytogenetic studies in *Asclepias* (Tourn.) L. Can. J. Research, C, **24**, 68–73.
24. MOORE, R. J. 1958. Cytotaxonomy of *Euphorbia esula* in Canada and its hybrid with *Euphorbia cyparissias*. Can. J. Botany, **36**, 547–559.
25. MULLIGAN, G. A. 1957. Chromosome numbers of Canadian weeds. I. Can. J. Botany, **35**, 779–789.
26. MULLIGAN, G. A. 1959. Chromosome numbers of Canadian weeds. II. Can. J. Botany, **37**, 81–92.
27. MULLIGAN, G. A. 1960. Polyploidy in Canadian weeds. Can. J. Genet. Cytol. **2**, 150–161.
28. MULLIGAN, G. A. The genus *Lepidium* in Canada. Madroño. In press.
29. PAL, N. *In* C. D. Darlington and A. P. Wylie, Ref. 5.
30. PETERSON, D. 1936. *Stellaria*–Studien. Zur Zytologie, Genetik, Ökologie und Systematik der Gattung *Stellaria*, insbesondere der *media*–Gruppe. Botan. Notiser, 281–419.
31. REESE, G. *In* C. D. Darlington and A. P. Wylie, Ref. 5.
32. RUTLAND, J. P. 1941. A list of chromosome numbers of British plants. New Phytologist, **40**, 210–214.
33. SARKAR, N. M. 1958. Cytotaxonomic studies on *Rumex* section Axillares. Can. J. Botany, **36**, 947–996.
34. SKOVSTED, A. 1935. Chromosome numbers in the Malvaceae I. J. Genet. **31**, 263–296.
35. STEBBINS, G. L. and PADDOCK, E. F. 1949. The *Solanum nigrum* complex in Pacific North America. Madroño, **10**, 70–81.
36. SUZUKA, O. 1950. Chromosome numbers in pharmaceutical plants. I. Rept. Kihara Inst. Biol. Research, **4**, 57–58.
37. SVENSCHNIKOVA, I. 1927. Die Genese des Kerns im Genus *Vicia*. Z. Induktive Abstammungs- u. Vererbungslehre, **2**, 1415–1421.
38. TARNAVASCHI, J. T. *In* G. Tischler, Ref. 40.
39. TATEOKA, T. 1954. Karyotaxonomy in Poaceae II. Somatic chromosomes of some species. Cytologia, **19**, 317–328.
40. TISCHLER, G. 1950. Die Chromosomenzahlen der Gefässpflanzen Mitteleuropas. Dr. W. Junk, 's-Gravenhage.

41. VAARAMA. *In* A. Löve and D. Löve, Ref. 17.
42. WIPF, L. 1939. Chromosome numbers in root nodules and root tips of certain Leguminosae. Botan. Gaz. **101**, 51–67.
43. WULFF, H. D. 1937. Chromosomenstudien an der schleswig-holsteinischen Angiospermenflora I. Ber. deut. botan. Ges. **55**, 262–269.
44. WULFF, H D. 1939. Chromosomenstudien an der schleswig-holsteinischen Angiospermenflora III. Ber. deut. botan. Ges. **57**, 84–91.

EVIDENCE FOR 'LIPOPEPTIDE', PHOSPHATIDYLETHANOLAMINE, AND PHOSPHATIDYLCHOLINE IN EXCISED TOMATO ROOTS[1]

W. G. Boll and A. A. Khan

Abstract

Evidence is presented for the existence of 'lipopeptide' in lipid extracted with ethanol: ether (3:1 v/v) from excised tomato roots grown in sterile culture. The amino acids released by acid hydrolysis of 'purified' lipid were identified by co-chromatography and color reaction with acidic and neutral ninhydrin reagent. They are cysteic acid, serine, glycine, aspartic acid, glutamic acid, glutamine, alanine, valine, leucine and/or isoleucine, and probably methionine and tyrosine. Arginine was detected on some chromatograms. Four unknown substances, three of which are ninhydrin-sensitive, were detected.

Paper chromatographic fractionation of water-soluble substances in methanolyzates of 'purified' lipid failed to yield a distinct 'peptide' fraction but fractions high in 'peptide' also contained two of the four unknown substances.

Glycerylphosphorylethanolamine and glycerylphosphorylcholine were detected in the methanolyzates. Glycerylphosphorylserine was not detected.

Introduction

This report stems from an investigation into methods for detecting and isolating ethanolamine, both free and combined, from excised tomato roots grown in sterile culture. During these studies it was found that acid hydrolyzates of a lipid fraction contained a number of nitrogenous compounds in addition to serine, ethanolamine, and choline which are known constituents of phospholipids.

Amino acids, other ionic substances, and sugars are frequently found in lipid extracts. Whether such substances are constituents or contaminants of the lipid is an old problem in lipid chemistry. In some cases the amino compounds are easily removed by washing, dialysis, or other mild treatments and they are presumably contaminants. In other cases, with lipid from animals or microorganisms, the purification methods have failed to remove the amino acids and for these and other reasons it has been claimed that amino acids, either as such or as peptide, may form part of lipid macromolecules. The terms 'aminolipid' and 'lipopeptide' have been introduced into lipid chemistry (22). Evidence for the view that excised tomato roots contain 'lipopeptide' is presented here.

Materials and Methods

Root Culture

The general techniques of root culture used here are described elsewhere (see Ref. 3 for leading references). The clone of roots used in all experiments is that designated R5. The isolation and nutritional requirements of this clone have been described (3). Root tips, 10 mm in length, excised from 4-day-old segments were grown in medium containing thiamine, pyridoxine, and niacin and were harvested on the sixth day.

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Lipid Extracts

The roots were killed in boiling absolute ethanol, and ether added to give a 3:1 v/v ratio of ethanol to ether. After homogenizing for 4 minutes in a Waring Blender the homogenate was filtered through sintered glass and the residue washed with solvent. The filtrate was concentrated under an air jet at room temperature. The subsequent procedure for preliminary purification by washing with water was as described by Kates and Eberhardt (13). This procedure, with extracts from other plants, has been found to remove water-soluble impurities effectively. The lipid residue so obtained is referred to as 'Total Lipid' (TL).

Acid Hydrolysis of Intact Lipid

The TL was hydrolyzed by refluxing with 3*N* HCl for 24 hours. The hydrolyzate was diluted with an equal volume of water and fatty acids removed with a mixture of petrol ether and ethyl ether (1:1). The aqueous phase was dried under the air jet. Residual HCl was removed by repeatedly adding distilled water and drying as described.

Mild Alkaline Hydrolysis (Methanolysis) of Intact Lipid

The procedure was based upon that described by Dawson (8). Purified lipid, equivalent to 250 roots, was dissolved in 15 ml of methanol and shaken for 5 minutes; 15 ml of 0.2*N* KOH in methanol was added and the mixture warmed at 38° C for 15 minutes, cooled immediately in ice-cold water, made up to 100 ml with the ice-cold water and passed through a column of IR 120 (H^+) resin (15 ml) previously cooled in the refrigerator. The column was washed, with 80 ml ice-cold water, into the effluent. This was then concentrated to small volume under an air jet and extracted twice with a mixture of petroleum ether – ethyl ether (1:1). The aqueous phase was then dried. In one experiment the resin column, after further washing, was eluted with 4% ammonium hydroxide and the eluate plus washings was dried.

Chromatography

Whatman No. 1 paper was used for all paper chromatographic procedures. In those experiments where material was eluted from the paper and rechromatographed either before or after hydrolysis, control experiments were made to determine the extent of interference or artifacts due to contaminants in the paper. These were found to be insignificant.

TABLE I

Solvent systems used for one-directional paper chromatography of total lipid

No.	Solvent	Direction
1	Water-saturated phenol (beaker of 0.3% ammonia in the chamber)	Descending
2	*n*-Butanol: acetic acid: water (4:1:5 v/v)	Descending
3	Water-saturated lutidine	Descending
4	Methanol: benzene: *n*-butanol: water (2:1:1:1 v/v)	Descending
5	*n*-Propanol: 0.25*N* aq. NH_4OH: acetone (4:1:1 v/v)	Ascending
6	Ether: ethanol: conc. NH_4OH (28%) (15:5:3 v/v)	Ascending
7	Methanol: benzene: *n*-butanol: water (2:1:1:1 v/v)	Ascending
8	Acetone: methanol (4:1 v/v)	Ascending
9	Water-saturated phenol	Ascending
10	Chloroform: ethanol: water (160:40:5 v/v)	Ascending

Chromatography of Intact Lipids

Paper chromatographic fractionation of TL was carried out with 10 different solvent systems which are given in Table I. The TL was applied to the paper in chloroform solution. The lipids were revealed with rhodamine B, or rhodamine G, coupled with viewing under ultraviolet light. Amino compounds were revealed with ninhydrin. Nonlipid bands were extracted with hot water. Lipid bands were removed, cut into small pieces, and extracted with chloroform for 24 hours in Soxhlet extractors. The lipid was then acid-hydrolyzed and aliquots were chromatographed in system 1 (Table II).

TABLE II

Paper chromatographic systems for hydrolyzates of total lipid

	Solvents	Time (hr)	Size of paper (in.)
System 1	1. Butanol: acetic acid: water (5:3:1 v/v)	12	16×13
	2. Butanol: ethanol: water (105:81:64 v/v)	13	
System 2	1. Water-saturated phenol (0.3% ammonia in the chamber)	33	22½×18½
	2. Butanol: acetic acid: water (5:3:1 v/v)	38	

Chromatography of Water-Soluble Products of Acid Hydrolysis

The paper chromatographic systems used for separation of the water-soluble acid-hydrolysis products are shown in Table II. System 1 is especially useful for the separation of serine, ethanolamine, and choline.

Amino compounds were detected by spraying either with a 0.25% solution of ninhydrin in acetone containing 4% collidine or with a 0.25% solution of ninhydrin in acetone containing 0.7% acetic acid. The former spray was used after acid solvents and the latter after neutral solvents. Phosphates were detected with a modified Hanes–Isherwood reagent (6); choline by spraying with 1% dipicrylamine in acetone–water (1:1:v/v) (15); sugars by spraying with either 5% ammoniacal silver nitrate (12), 1% *p*-anisidine hydrochloride in ethanol (16), or naphthoresorcinol in ethanol (9). Inositol and glycerol were also revealed by the ammoniacal silver nitrate. Bromcresol green spray was used to detect acid areas.

Chromatography of the Water-Soluble Products of Methanolysis

The water-soluble products of methanolysis were chromatographed in system 1 or were fractionated by banding the extract and running in one dimension using butanol: ethanol: water (105:81:64 v/v). Recognized bands were then eluted and, when necessary, were run again using water-saturated phenol. The details of this fractionation are given below.

Electrophoresis of Emulsions of Intact Lipid

Paper electrophoresis of emulsions of intact lipid was carried out by the method of Westley *et al.* (20) using a Durrum-type cell, phthalate buffer at pH 4.15, and a current of 500 volts for 6 hours or 200 volts for 14 hours. In addition, emulsions placed in a U tube, and overlayered with buffer, were subjected to an electric current. The details are given below.

Concentration of Extracts

All extracts and fractions thereof were concentrated or dried under air jets either at room temperature, with extracts in volatile organic solvents, or at a temperature not exceeding 60° C with aqueous solutions. It is recognized that the use of an air jet may lead to peroxidation and the possible formation of artifacts. However, the type of artifact expected would not be such as to invalidate the conclusions reached here.

Experimental and Results

The Constituents in Acid Hydrolyzates of Total Lipid

Figure 1 shows the ninhydrin-sensitive areas on a chromatogram (system 1) of an acid hydrolyzate of TL. The positions of serine, ethanolamine, and leucine are indicated. A number of other ninhydrin-sensitive areas are visible and these occurred on all such chromatograms. Most of them are amino acids but there are also three unknown amino compounds. Two of these are the fast spots U1 and U2. The other, U3–U4, interferes with glutamic acid. It reacts with ninhydrin to give a reddish-violet color which fades more rapidly than the amino acid spots. U3–U4 is acid and on one chromatogram using system 2 the spot

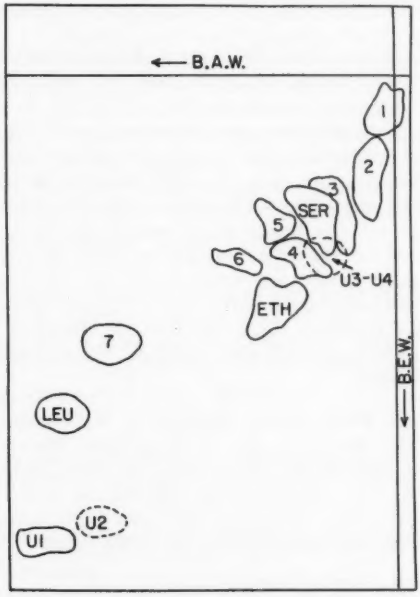

FIG. 1. Ninhydrin-positive spots on a chromatogram of the acid hydrolyzate of total lipid. Spots U1 to U4 are unidentified. The positions of serine (SER), ethanolamine (ETH), and leucine (LEU) are shown. The other spots are: 1, cysteic acid; 2, arginine; 3, aspartic acid; 4, glutamic acid; 5, glutamine; 6, alanine; and 7, valine–methionine. Glycine overlaps the serine and aspartic acid spots. Tyrosine was detected when larger quantities of hydrolyzate were used.

was resolved into an acid spot and a ninhydrin-sensitive spot. Various color tests indicated that U3 and U4 do not correspond to any known constituent of plant lipid hydrolyzates. Thus there are at least four unidentified, and possibly novel, compounds present.

Attempts to Remove the Amino Compounds from Lipid Extracts

Generally speaking, the presence of aminolipid or lipopeptide is concluded from the fact that the amino acid (or acids) is not removed even by extensive purification using methods expected to remove amino acid or peptide. It is largely, but not solely, such negative evidence which we now have for the existence of lipopeptide in the excised tomato root. This evidence is given below.

TL was subjected to various conventional and less-known methods to remove amino acids. They were not removed by washing vigorously and repeatedly with many volumes of distilled water, even though chromatography of the concentrated aqueous phase showed the presence of free amino acids. Dialysis of emulsions even for as long as 15 days was ineffective although the concentration of amino acids was decreased. The decrease was not uniform between amino acids and, in view of the prolonged dialysis, could have been due to decomposition of lipid. Chromatography of lipid on cellulose columns will remove amino acids from lipid from either human serum (7) or plant material (13, 18). This method, using the technique of Cheftel *et al.* (7), was ineffective with TL from the roots even when followed by prolonged dialysis.

Paper chromatographic fractionation of intact lipid (see Table I) removed free amino acids from unwashed lipid and also removed amino acids added to emulsions of TL prior to application on the paper. However, the usual amino compounds still appeared in acid hydrolyzates of lipids eluted from the paper. With each of the solvent systems shown in Table II the lipids gave one band, of varying width, at the solvent front. After staining with rhodamine G two adjoining bands were distinguishable within the lipid area. The band with the lower R_f value also stained with ninhydrin. This partial resolution of lipid was most prominent with the solvent number 10 (Table I). Subsequently, this solvent was used routinely to give a lipid preparation which will be referred to as 'purified lipid' (PL).

In a further attempt to purify lipid by paper chromatography, samples of TL were run in each of the solvent systems numbers 5, 6, 7, 8, and 9 shown in Table I. The lipid areas were eluted and rechromatographed separately using solvent 10. Chromatograms (system 1) of acid hydrolyzates of the resulting lipid samples still showed the typical pattern of amino compounds.

Hydrolysis of the two bands distinguishable in the lipid areas showed that the ethanolamine and choline were detectable only in the slower, ninhydrin-sensitive band. Both bands yielded the typical pattern of amino acids. Thus the amino acids are not associated solely with phosphatidyl-ethanolamine and -choline. The most important point, however, is that lipid areas which were not ninhydrin-sensitive still yielded amino acids on hydrolysis.

The amino acids present in PL were identified using principally chromatographic system 2 and larger quantities of hydrolyzate than was used for the chromatograms illustrated in Figs. 1 and 3. On the basis of position, co-chromatography, and color reaction of the spots with neutral or acidic ninhydrin

reagent, the amino acids are believed to be cysteic acid, serine, glycine, aspartic acid, glutamic acid, glutamine, alanine, valine, leucine and/or isoleucine, and probably methionine and tyrosine. Arginine was detected on some chromatograms.

A further group of experiments involved attempts to remove amino acids, or other charged molecules containing amino acids, from PL by electrophoretic methods. Westley *et al.* (20) showed that amino acids, lactams of γ-aminobutyric and δ-aminovaleric acids, and small cyclic peptides, which are not separated from lipids by chromatography, are separated from lipids by paper electrophoresis of emulsions of *Drosophila* lipids. They claimed, perhaps extravagantly, that paper electrophoresis permits a clear distinction between bound amino acids and those rendered soluble by phospholipids. When the method of Westley *et al.* was applied to lipid from the tomato roots we found that free amino acids were removed from TL and, using PL, we confirmed that amino acids added to the lipid, and emulsified with it, are separated by electrophoresis. However, it was clearly shown that the typical pattern of amino compounds was still present in hydrolyzates of PL which had been subjected to electrophoresis.

An alternative electrophoretic method was devised. It was suggested by claims (4) that nitrogenous substances have been removed from emulsions of lipids by electrodialysis. The presence of a membrane is a possible disadvantage if the amino compounds are part of large, nonlipid molecules. Hence, the PL, emulsified in buffer, was placed in a U tube and overlayered with buffer (phthalate, pH 4.35). Filter-paper strips were placed between the arms of the U tube and the reservoirs of buffer containing the electrodes. A current of 800 volts, 1.4–2.4 ma, was passed. This technique failed because the emulsions broke and the current stopped. As an alternative the emulsion was placed in a thin layer in the cross arm of the U tube. A current of 800 volts, 1.4–2.4 ma, was passed for 16 hours while the buffer was changed at intervals. It was assumed that any charged compounds at the surface of the lipid phase would be removed. Although this technique gave some decrease in the amino acids in TL it did not eliminate them from PL.

Fractionation of Methanolyzates of Purified Lipid

As an attempt to obtain more definitive evidence for amino acid containing lipid than that given above, the products of methanolysis of PL were examined.

If lipids are subjected to a relatively short treatment with methanolic alkali, the lipids containing glycerol are cleaved at the bond, or bonds, between glycerol and the fatty acids. The products can then be separated into water-soluble, glycerol-containing components and the fatty acids which are soluble in petrol–ether or other suitable solvent. The importance of this reaction here is that with the disruption of the macromolecule we could expect the release of any adsorbed amino acids or peptide. These could then be recovered in the free form in a water-soluble fraction. On the other hand, amino acids forming integral parts of the lipid or peptide might only be released on further acid hydrolysis. Amino-acid-containing moieties could be isolated from the methanolyzate.

PL, equivalent to 250 roots, was subjected to methanolysis as described

above. The effluent, which is referred to as 'methanolyzate', and the material eluted from the resin column were then examined separately.

Methanolysis Products Adsorbed on IR 120 (H^+) Resin

An aliquot of the eluate was chromatographed (system 1) and shown to contain free amino acids. An equivalent aliquot was chromatographed after acid hydrolysis. As compared with the chromatogram of the unhydrolyzed aliquot, there was a slight increase in the intensity of some ninhydrin-sensitive spots but none were eliminated.

Some ethanolamine was detected in hydrolyzates of the eluate. However, the bulk of the ethanolamine in the methanolyzate was in the original effluent.

Methanolysis Products Not Adsorbed on IR 120 (H^+) Resin

An aliquot of the effluent, equivalent to 63 roots, was chromatographed (system 1) and stained with ninhydrin. The chromatogram was strikingly different from that of the acid hydrolyzate of PL. It showed one spot of high intensity occupying a position close to that of serine shown in Fig. 1. In addition some faint spots were detected. A chromatogram of a corresponding aliquot, after acid hydrolysis, showed a high concentration of ethanolamine and the typical pattern of intense, ninhydrin-sensitive spots found on the chromatograms of the acid hydrolyzates of PL. Because of the position of these spots it was not possible to decide whether any of the faint ninhydrin-sensitive spots on the chromatogram of the unhydrolyzed aliquot had disappeared. Nevertheless, the methanolyzate undoubtedly contained conjugated amino acids either in peptide or in some other form. The term 'peptide' is used here, and subsequently, in a generic sense and with reservation.

Clearly, if it could be shown that the conjugated amino acids formed part of a moiety containing residues other than amino acids, and preferably including glycerol, then this would be strong evidence for a lipopeptide. Accordingly, a fractionation of the methanolyzate was attempted.

Further Paper Chromatographic Fractionation of Methanolyzate

Methanolyzate from 250 roots, after being passed over IR 120(H^+) resin and extracted with petroleum ether: ethyl ether as described above, was concentrated and streaked along a 6-in. line on a piece of Whatman No. 1 paper measuring 8 in. × 45.5 cm. The chromatogram was run with butanol: ethanol: water (105:64:81 v/v), descending, for 18 hours. After drying, longitudinal strips were taken from the center and sprayed with one of the following reagents: either ninhydrin, ammoniacal silver nitrate, Burrow's molybdic acid reagent for phosphates (6), or periodate-Schiff's reagent (5) for the detection of glyceryl glycosides. The positions of the bands stained by these reagents are shown in Fig. 2. On the basis of these tests the remainder of the chromatogram was divided into five bands as indicated in Fig. 2. Each of the bands was extracted separately with hot water and dried. The resulting residues were hydrolyzed with acid and appropriate aliquots were chromatographed (system 1).

Figure 3 shows the chromatograms, stained with ninhydrin, of the unhydrolyzed and hydrolyzed aliquots of the No. 3 band. The unhydrolyzed aliquot contained the spot of high intensity which was detected on chromatograms of

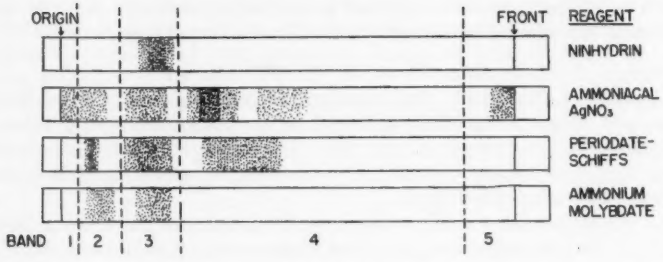

FIG. 2. Chromatography of water-soluble products in the methanolyzate of purified lipid showing the position of bands revealed by the reagents given on the right.

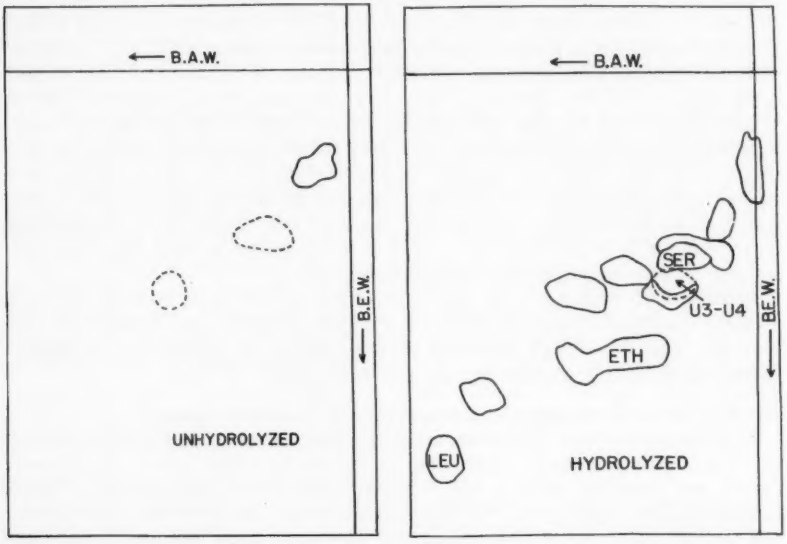

FIG. 3. Ninhydrin-positive spots on chromatograms of material eluted from band 3 of the chromatogram illustrated in Fig. 2. The extract on the right was further hydrolyzed with acid.

the complete methanolyzate. The hydrolyzed aliquot contained ethanolamine and gave the typical pattern obtained with acid hydrolyzates of PL. Such hydrolyzates also contained choline, phosphate, and glycerol.

Corresponding chromatograms of bands 1, 2, 4, and 5 all showed amino compounds, other than ethanolamine, in the hydrolyzed aliquots. Furthermore, there was little difference between the patterns obtained with the different bands. It seems that the same 'peptide' was distributed all over the original chromatogram although inspection of chromatograms of the five hydrolyzates showed some concentration of 'peptide' in bands 3 and 5. These were narrow

bands on the paper. Furthermore, the acid–ninhydrin-positive spot (U3–U4) was also prominent in both of these bands.

A further fractionation of band 3 was carried out using lipid from 315 roots. Band 3 was extracted with hot water and the concentrated extract was transferred to a second chromatogram of the same dimensions as the first one. This was run with water-saturated phenol, descending, for 16 hours. The solvent is known to separate glycerylphosphorylethanolamine (GPE) from glycerylphosphorylcholine. Guide strips were removed and sprayed either with ninhydrin, ammoniacal silver nitrate, or Burrow's molybdic acid reagent. Again, five contiguous bands, designated 1*a* to 5*a*, were delineated. Bands 1*a* and 5*a* corresponded in width to bands 1 and 5 shown in Fig. 2. Band 3*a* was ninhydrin-sensitive. It corresponded in width to band 3 of Fig. 2 but had an R_f of about 0.64.

The five bands were extracted separately with warm water, hydrolyzed with acid, and aliquots chromatographed (system 1). Band 3*a* contained ethanolamine and band 5*a* contained choline; hence the ethanolamine- and choline-containing moieties are distinct and effectively separated by the phenol solvent. Bands 3*a* and 5*a* also contained glycerol and phosphate. However, each of the bands again, with minor variations in concentration, gave the pattern of amino compounds found with acid-hydrolyzed PL. The concentrations of leucine and the valine/methionine spot were relatively high in bands 2 and 5. All amino spots in band 5*a* were more intense than with other bands. The U3–U4 spot was concentrated in bands 3*a* and 5*a* with some in band 1*a*.

In a separate experiment, methanolyzate was chromatographed together with an authentic sample of GPE kindly supplied by Dr. Erich Baer of the University of Toronto. On one-dimensional chromatograms, using either butanol: ethanol: water (105:81:64 v/v) or water-saturated phenol as solvent, the ethanolamine-containing fraction travelled to the same position as the authentic GPE.

Obviously, a single 'peptide' fraction cannot be isolated by the above chromatographic methods. The results suggested that either a single 'peptide' or a number of closely related 'peptides' with ill-defined R_f values were present or that some 'peptide'-containing moiety or moieties were decomposing during chromatography. Accordingly a further experiment involving rechromatography was carried out.

The experiment, involving rechromatography, just described was repeated, using methanolyzate equivalent to 222 roots, except that the separate extracts from bands 1*a*, 3*a*, and 5*a*, of the phenol chromatogram of band 3, were rechromatographed with phenol. Chromatography (system 1) of the acid hydrolyzates of material extracted from bands corresponding to bands 1*a*, 3*a*, and 5*a* of the first phenol run showed that 'peptide', and significantly U3–U4, was present in all three fractions. Thus the 'peptide' moieties from bands 1*a*, 3*a*, and 5*a* in the first phenol run did not simply reoccupy the same positions on rechromatography in the same solvent although, from inspection, it appeared that the greatest concentration of each 'peptide' fraction was in the same position on rechromatography. The ethanolamine-containing moiety and choline-containing moiety occupied the same position on rechromatography as in the first phenol run.

Discussion

This work clearly demonstrates that the lipid extracted from excised tomato roots contains amino acids in organic combination. There is not proof that they are in the form of peptide although this is most likely to be the case. For convenience it is assumed that peptide is present. Rigorous proof of the existence of a 'lipopeptide' requires the isolation of a distinct compound of constant chemical composition. This must await the perfection of techniques for the fractionation of lipids. Nevertheless, we feel that the most likely interpretation of the observations reported here is that excised tomato roots contain 'lipopeptide'. The reasoning is as follows and is, to our knowledge, the only published evidence for 'lipopeptide' in plant tissue other than storage tissue (cf. Ref. 20).

Amino acids were detected in acid hydrolyzates of the lipid even after extensive purification procedures. These included methods by which free amino acids, either in the original lipid or added to emulsions, were removed into nonlipid fractions. A lipid fraction purified by paper chromatography did not stain with ninhydrin but gave free amino acids on hydrolysis whereas the ethanolamine-containing lipid stained readily with ninhydrin. Hence the amino acids are either bound through the amino groups or are in the form of 'peptide', perhaps cyclic, giving no or slight ninhydrin reaction. The former explanation seems the less likely.

The occurrence of 'peptide' in intact lipid fractions, after various purification techniques, gives only negative evidence for the presence of lipopeptide. Chromatographic fractionation of the methanolyzate of PL was somewhat more instructive. The solvents used for the paper chromatographic fractionation described here are expected from the work of others (10, 11, 14) to give distinct separation of certain peptides although Synge (19) has stated that peptides, except those of low molecular weight, streak badly. On our chromatograms there was some concentration of 'peptide' in certain bands but it was detected over the whole 'butanol' or 'phenol' chromatogram. Furthermore, when limited areas of the 'phenol' chromatogram of band 3 were rechromatographed in aqueous phenol the 'peptide' was again distributed over the whole chromatogram. This distribution was not a consequence of general interference because other residues, such as those containing ethanolamine or choline, were effectively resolved and were restricted to narrow bands.

Two explanations of the distribution of the 'peptide' are suggested. One is that the 'peptide' is a distinct compound but streaks badly. The second is that the distribution is a consequence of the decomposition of larger peptide-containing moieties. This latter view is supported by the fact that, following rechromatography of one particular band in the same solvent, the 'peptide' was redistributed over the entire paper but was concentrated in at least *two* bands. Furthermore, on the first 'phenol' chromatograms the high 'peptide' bands always gave the unknown spot (U3–U4) detected in acid hydrolyzates; on rechromatography of one high 'peptide' band (band 3) in 'phenol' the *two* high 'peptide' bands which resulted both also gave high concentrations of U3–U4 in the acid hydrolyzates. These results suggest that the 'peptide' and U3–U4 are in organic combination.

The ethanolamine- and choline-containing residues in the methanolyzate had the same R_f value in the 'butanol' solvent but were separated by aqueous phenol. The fractions so obtained also contained glycerol and phosphate. The R_f values of glycerylphosphorylethanolamine (GPE) and glycerylphosphorylserine (GPS) in phenol: water (4:1 w/w) are 0.67 and 0.12 respectively (1). The value for GPE agrees closely with that for the ethanolamine-containing fraction obtained here (R_f 0.64). Furthermore, in both the 'butanol' solvent and aqueous phenol the ethanolamine-containing fraction travelled to the same position as an authentic sample of GPE. It is concluded that the lipid contains GPE. No evidence for the presence of GPS was obtained here. Other workers have also failed to detect GPS in plant lipid (cf. Refs. 3, 21).

The movement of glycerylphosphorylcholine (GPC) relative to GPE on chromatograms run in aqueous phenol was calculated from chromatograms illustrated by Benson and Maruo (2). If GPE is at 1.0, then GPC is at about 1.4. Our value for the choline-containing fraction is 1.4 to 1.5. Acid hydrolysis of this fraction gave glycerol and phosphate; hence it is concluded that the lipid contains GPC.

The bulk of the ethanolamine present in the lipid was recovered as GPE which was not adsorbed on the column of IR 120 (H^+) resin used to neutralize the methanolyzate. Benson and Maruo (2) have implied that some GPE is adsorbed on such resin. This may account for the fact that here some ethanolamine was detected in acid hydrolyzates of the eluate from the resin. Nevertheless the possibility cannot be ignored that there is an ethanolamine-containing residue, other than GPE, in the lipid.

References

1. ANSELL, G. B. Private correspondence.
2. BENSON, A. A. and MARUO, B. 1958. Plant phospholipids. I. Identification of the phosphatidylglycerols. Biochim. et Biophys. Acta, **27**, 189–195.
3. BOLL, W. G. 1959. Evidence for negative feedback in the control of ethanolamine biosynthesis in excised tomato roots. Can. J. Botany, **37**, 1071–1083.
4. BLASS, J., ROULIN, A., LECOMTE, O., and MACHEBOEUF, M. 1953. Recherches sur les constituents azotés des extraites lipidiques du sang. Bull. soc. chim. biol. **35**, 959–968.
5. BUCHANAN, J. G., DEKKER, C. A., and LONG, A. G. 1950. The detection of glycosides and non-reducing carbohydrate derivatives in paper partition chromatography. J. Chem. Soc. 3162–3167.
6. BURROWS, S., GRYLLS, F. S. M., and HARRISON, J. S. 1952. Paper chromatography of phosphoric esters. Nature, London, **170**, 800–801.
7. CHEFTEL, R. I., MORETTI, J., and POLONOVSKI, J. 1957. Études des peptides associés aux phospholipides du sérum humain. Bull. soc. chim. biol. **39**, 291–299.
8. DAWSON, R. M. C. 1954. The measurement of P^{32} labelling of individual kephalins and lecithins in a small sample of tissue. Biochim. et Biophys. Acta, **14**, 374–379.
9. DEDONDER, R. 1952. Les glucides de Topinambour: I. Mise en evidence d'une série de glucofructosans dans les tubercules du Topinambour. Isolement, analyse, et structure des termes les moins polymerisés. Bull. soc. chim. biol., Paris, **34**, 144–170.
10. DOURMONT, Y. P. and FRUTON, J. S. 1952. Chromatography of peptides as applied to transamidation reactions. J. Biol. Chem. **197**, 271–283.
11. HANES, C. S., HIRD, F. R. J., and ISHERWOOD, F. A. 1952. Enzymatic transpeptidation reactions involving γ-glutamyl peptides and α-amino-acyl peptides. Biochem. J. **51**, 25–35.
12. HOUGH, L. 1950. Application of paper partition chromatography to the separation of polyhydric alcohols. Nature, **165**, 400.
13. KATES, M. and EBERHARDT, F. M. 1957. Isolation and fractionation of leaf phosphatides. Can. J. Botany, **35**, 895–905.

14. KATZ, A. M., DREYER, W. J., and ANFINSEN, C. B. 1959. Peptide separation by two dimensional chromatography and electrophoresis. J. Biol. Chem. **234**, 2897–2900.
15. MALYOTH, G. and STEIN, H. W. 1951. Beitrag zur Papierchromatographie der Cholinester und der Zucher. Biochem. Z. **322**, 165–167.
16. MUKHERJEE, S. and SRIVASTAVA, H. C. 1952. Improved spray reagent for the detection of sugars. Nature, London, **169**, 330.
17. RONSER, G., MARINETTI, G., and BERRY, J. F. 1954. Paper chromatography of phospholipids. Federation Proc. **13**, 286.
18. SMITH, R. A. 1954. The phosphatides of the latex of *Hevea brasiliensis*. 2. Purification and analysis. Biochem. J. **57**, 130–139.
19. SYNGE, R. L. M. 1955. Peptides (bound amino acids) and free amino acids. *Edited by* K. Paech and M. V. Tracey. *In* Modern methods of plant analysis. Vol. IV. pp. 1–22.
20. WESTLEY, J., WREN, J. J., and MITCHELL, H. K. 1957. Phospholipides containing amino acids other than serine. J. Biol. Chem. **229**, 131–138.
21. WINTERMANS, J. F. G. M. 1960. Concentrations of phosphatides and glycolipids in leaves and chloroplasts. Biochim. et Biophys. Acta, **44**, 49–54.
22. WREN, J. J. 1960. Lipoamino-acids: the question of artifact. Nature, London, **185**, 295–297.

THE LEPTOGRAPHIUM COMPLEX. PHIALOCEPHALA GEN. NOV.[1]

W. Bryce Kendrick

Abstract

Some *Leptographium*-like hyphomycetes are maintained as distinct from that genus on the basis of spore ontogeny, and a new genus, *Phialocephala*, erected for them.

This paper is the first of a series comprising a taxonomic treatment of a poorly known but important group of hyphomycetes, which are often associated with disease in forest trees, and have usually been lumped together under the generic name *Leptographium* Lagerb. and Mel. (1928). This complex of fungi is characterized by the possession of a highly evolved, darkly pigmented, often massive conidiophore, in which a mononematous stipe bears at its apex a sporogenous apparatus consisting of one to several multiplicative series of parallel or slightly divergent metulae. Each metula of one series bears from one to four of the metulae comprising the next higher series, and each of the ultimate metulae bears sporogenous cells in the same ratio. Thus, assuming the multiplication factor of each series to be approximately three, a conidiophore with five series of metulae will bear about 3^6 or 729 sporogenous cells. Each sporogenous cell may produce a considerable number of more or less hyaline amerospores, and the products of all the sporogenous cells accumulate round the sporogenous apparatus in a milky drop of slime. A single conidiophore may produce many thousands of spores, which are presumably distributed by the various small animals which come in contact with the spore drop.

It has become clear from examination of the type species of *Leptographium*, *L. lundbergii*, that the sporogenous cells of this species are annellophores, and that this genus therefore belongs to Section III of the experimental classification proposed for the hyphomycetes by Hughes (1953). The fungi considered in this paper, while possessing a conidiophore very similar in construction and general appearance to that of the true *Leptographium*, have been found to produce phialides as their sporogenous cells instead of annellophores. This places these fungi in Section IV of Hughes' scheme, and the erection of a separate genus for such phialide-bearing species becomes necessary.

Phialocephala Kendrick gen. nov.

Fungi imperfecti, hyphomycetes dematiaceae.

Conidiophorae erectae, solitariae vel raro fasciculatae. Stipites mononematei, dematiacei. Apparatus sporogenus obconicus complexus in apice stipitis natus, ex seriebus 1-pluribus metularum, distaliter phialides ferentes. Phialides saepius strophium gaudentes. Phialosporae parvae, unicellulariae,

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saepius hyalinae, ex phialidibus in capitulum mucosum successive extrusae.

Habitat: Saepius in ligno et cortico putrescentibus, vel isolata ex ligno condito vel arboribus viventibus.

Species typica: *P. dimorphospora* Kendrick sp. nov. (vide infra).

Other species: *P. bactrospora* Kendrick sp. nov. (described below).

Fungi imperfecti, dematiaceous hyphomycetes. Conidiophores are darkly pigmented, solitary or occasionally clustered, with a single stipe bearing at its apex a complex sporogenous head. This consists of from one to several multiplicative series of metulae, the ultimate series of which collectively bear numerous phialides. The phialides often possess well-marked collarettes, and produce numerous small, usually hyaline, amerospores, which often become aggregated around the sporogenous head in a drop of slime.

Habitat: Often on decaying wood or bark, or isolated from worked wood or living trees.

P. dimorphospora has been chosen as the type species because of its unmistakeable phialides and its well-differentiated conidiophore.

Phialocephala dimorphospora Kendrick sp. nov.

In sylvestris, **conidiophorae** inconspicuae, erectae, sparsae, raro fasciculatae. **Stipites** mononematei, parietibus crassis, basi fusci, apicaliter pallescentes, 2–5-septati, 19–81 μ long., 6.7–10.1 μ crass. ad basas, 4.9–6.7 μ ad summas. **Caput sporogenum** obconicum complexum, 30–70 μ long., in apice stipitis natum, ex seriebus 2–5 metularum distaliter ferentes phialides. **Metulae** primariae saepius geminae, pariete crasso, cum stipite concolores, 8.0–16.2×3.5–5.4 μ. Metulae secundariae saepius 3–7, pariete tenui, pallidioribus, 8.0–13.5×2.7–3.8 μ. Tertiariae saepius 15–20, subhyalinae, 6.7–13.5× 2.4–3.2 μ. Quaternariae plurimae, hyalinae, 5.4–9.4×2.2–3.0 μ. **Phialides** compactae, hyalinae, subcylindraceae, ad medias crassiores, ad summas strophium elongatum cylindricumque gaudentes. Strophium 3.8–5.4×2.3–2.8 μ. Phialides 10.8–17.5×2.5–3.1 μ. **Phialospora** primaria ovoidea, 3.6–5.4×2.3–2.8 μ, alterae subsphericae 1.7–2.4×1.7–2.4 μ. Phialosporae laeviae, hyalinae, glutinosae, in catenis pertinacibus extrusae, et in capitulum mucosum congregantes.

In vitro, coloniae lente crescentes, diam. aetate 15 dierum 4 cm in agaro 'malt' dicto, 3.5 cm in agaro 'potato-dextrose' dicto. Vitrum mensibus duobus tectum. Coloniae in ambis solis consimiles aspectu. Mycelium aerium coactile, a 'deep mouse gray'[2] 'dark mouse gray'. Colonia in tergo 'fuscous black'. Margo subdiffusa, minute striata. Hyphae fuscae, irregulariter sinuosae, a glabris perrugosae, saepius 3.5–5.0 μ crass. Conidiophorae majores ad 88 μ long., inconspicuae, ex medio extus natae, minores ex hyphis aeriis natae. Conidiophorae si in vitro minus differentiatae quam in solo, sed phialides phialosporaeque, parum si diversae, phialides 9.4–18.0×2.4–4.0 μ. Phialospora primaria 4.6–6.4×1.9–2.7 μ, alterae 2.0–2.7×2.0–2.6 μ. Apparatus sporogenus in primis subhyalinus, deinde stipite colorato, in aetate omnino coloratus.

[2]Colors cited from R. Ridgway, "Color standards and color nomenclature" (Washington, 1912).

Typus: On rotten deciduous wood, one mile south of Manotick, Ontario. November 1960. S. J. Hughes. DAOM 71465(c).

Other collection examined: On rotten deciduous wood, Gatineau Park, Province of Quebec. November 1960. S. J. Hughes. DAOM 71381(b).

On the natural substrate the very inconspicuous conidiophores are erect, scattered or rarely in small clusters. The mononematous stipe is thick-walled

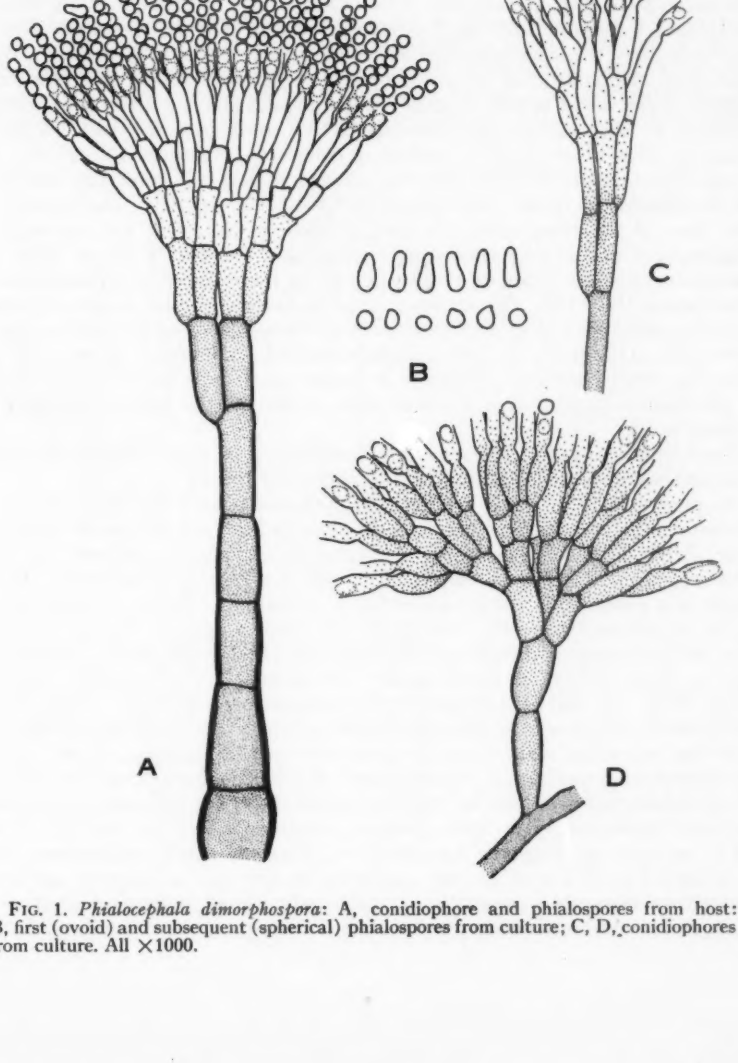


FIG. 1. *Phialocephala dimorphospora*: A, conidiophore and phialospores from host: B, first (ovoid) and subsequent (spherical) phialospores from culture; C, D, conidiophores from culture. All ×1000.

and fuscous at the base, gradually becoming paler toward the apex. It is 2–5 septate, 19–81 μ long, 6.7–10.1 μ thick at the base, and 4.9–6.7 μ thick near the apex. The complex obconical sporogenous head produced at the apex of the stipe (Figs. 1A, 5, 6, 8) consists of 2–5 series of metulae ultimately bearing a cluster of phialides. The total length of the sporogenous head ranges from 30 to 70 μ. The primary metulae usually number 2 and measure 8.0–16.2×3.5–5.4 μ; they are thick-walled and almost concolorous with the stipe. Secondary metulae, commonly 3–7 in number, are thinner-walled and paler, measuring 8.0–13.5×2.7–3.8 μ. Tertiary metulae, commonly 15–20 in number, are subhyaline, and measure 6.7–13.5×2.4–3.2 μ. The numerous quaternary metulae are hyaline, and are 5.4–9.4×2.2–3.0 μ. The phialides all arise at approximately the same level and are consequently packed together in a tight fascicle at the summit of the conidiophore. They are hyaline, subcylindrical, slightly wider in the middle than at either end, and above a distal construction develop a long cylindrical collarette which measures 3.8–5.4×2.3–2.8 μ (Fig. 5). The entire phialide, including collarette, measures 10.8–17.5×2.5–3.1 μ. The first phialospore, which is formed within the unbroken extension of the phialide, is ovoid, measuring 3.6–5.4×2.3–2.8 μ. After the rupture of the apex of the outer wall, the first phialospore is freed, and subsequent phialospores are almost spherical, measuring 1.7–2.4×1.7–2.4 μ. This accounts for the two types of phialospore to be found in slide preparations of this species (Fig. 1B). The spherical type is naturally much more numerous than the ovoid type (Fig. 7). Up to three of the spherical spores may be found within the collarette of an active mature phialide, and spore chains observed emerging from phialides commonly comprise up to nine conidia. The chains of phialospores are slimy and accumulate at the apex of the conidiophore in a slimy head.

Both collections of *P. dimorphospora* were made on rotten deciduous wood, and both were successfully isolated on laboratory media.

In pure culture at room temperature, colonies spread slowly, attaining a diameter of 4 cm after 5 days on malt agar and 3.5 cm on potato dextrose agar. After 2 months, however, the plate is completely covered. General appearance of colonies on malt and potato dextrose agars is very similar. There is a moderately thick turf of 'deep mouse gray' to 'dark mouse gray' aerial mycelium, while the reverse of the colony appears 'fuscous black'. The margin is slightly diffuse and finely striate. Hyphae are brown, irregularly sinuous, smooth to quite rough-walled, and usually 3.5–5.0 μ wide. Conidiophores (Figs. 1 C and D, 3, 4) are very inconspicuous in the floccose mycelium. Those forming on aerial hyphae are rather reduced, and those arising directly from the substrate, while more complex and up to 88 μ long, do not attain the degree of organization characteristic of conidiophores found in nature. The phialides and phialospores, however, retain their characteristic appearance, and their dimensions are little changed, phialides measuring 9.4–18.0×2.4–4.0 μ, primary phialospores 4.6–6.4×1.9–2.7 μ, and other phialospores 2.0–2.7×2.0–2.6 μ. The sporogenous apparatus ranges with advancing age from subhyaline, through a condition in which only the stipe is pigmented, to a final generalized brown coloration of all but the tips of the collarettes.

The second species of *Phialocephala* has not been found fruiting on wood, but has been obtained in pure culture twice, in isolations derived from stained *Tilia* veneers in England, and from *Populus trichocarpa* in Canada.

Phialocephala bactrospora Kendrick sp. nov.

Ex culturis in agaro descripta.

Coloniae radiantes, diam. aetate 15 dierum 4.8 cm in agaro 'malt' dicto, 5.6 cm in agaro 'potato dextrose' dicto. In agaro 'malt' dicto mycelium aerium 'chaetura drab', laxe floccosum, densior ad medias, laxissimum ad marginem diffusissimam. **Conidiophorae** plurimae ex medio extus et ex hyphis aeriis natae. **Stipites** mononematei a fuscis, pallide fuscis, pariete tenue, multiseptati, haud attenuati, 460–1060 μ long., 4.0–7.0 μ crass. **Apparatus sporogenus** in apice stipitis natus, 38–90 μ long., ex 1–2 seriebus metularum, cum stipite concolores, interdum irregulariter dispositae, atque distaliter phialides ferentes. **Phialides** pallidiores, strophium elongatum

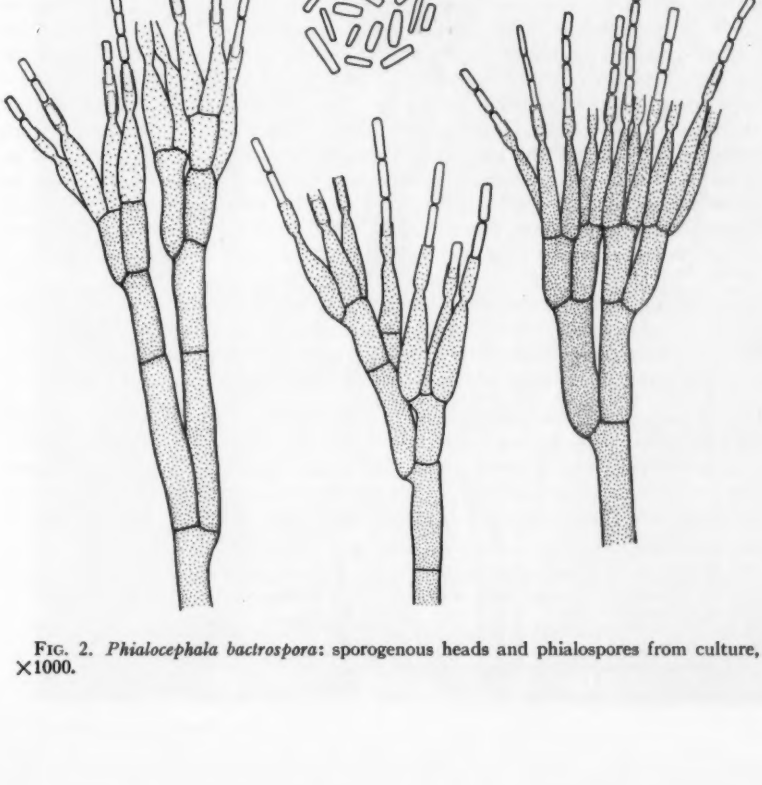


FIG. 2. *Phialocephala bactrospora*: sporogenous heads and phialospores from culture, ×1000.

cylindricumque gaudentes. Metulae primariae 16–48×3.6–5.6 μ, metulae secundariae 9.8–16.8×3.2–4.6 μ. Phialides 16.2–27.6×3.5–4.3 μ, strophium 4.7–7.5×1.8–2.2 μ. **Phialosporae** bacilliformae, hyalinae, 3.2–10.6×0.7–1.7 μ, in catenis mucosis pertinacibus extrusae. Phialosporae omnes, et primaria et alterae, consimiles. Coloniae si in agaro 'potato dextrose' dicto gaudent mycelium aerium sterile copiosus et conidiophorae perpaucae quam si in agaro 'malt' dicto.

Typus: Isolated from *Populus trichocarpa*, Cinema, British Columbia, J. Foster, 1949. Dried cultures DAOM 28736, also Herb. IMI 47572.

Other collection examined: Isolated from stained *Tilia* veneers, Wisbech, England, J. G. Savory, August 1952. Herb. IMI 52633, DAOM 63900.

At room temperature colonies are spreading, reaching a diameter of 4.8 cm after 15 days on malt agar and 5.6 cm on potato dextrose agar. On malt agar the 'chaetura drab' aerial mycelium is loosely floccose, being most dense in the center and gradually becoming scantier toward the margin, which is very diffuse. Some fine sterile hyaline aerial mycelium is often found overgrowing the aerial mat of darker hyphae. Numerous conidiophores arise both from the surface of the medium and from the aerial mycelium. The mononematous stipe is medium to light fuscous, thin-walled, polyseptate, from 460 to 1060 μ long, not tapered and 4.0–7.0 μ thick. The sporogenous head (Figs. 2, 11) borne at the apex of the stipe consists of 1–2 series of metulae sometimes rather irregularly arranged and usually concolorous with the stipe, and a distal cluster of characteristic phialides which are usually paler in color and possess a long cylindrical collarette (Figs. 9, 10, 11). The total length of the sporogenous head ranges from 38 to 90 μ. The primary metulae measure 16–48×3.6–5.6 μ, and the secondary metulae measure 9.8–16.8×3.2–4.6 μ. The entire phialides, including collarettes, measure 16.2–27.6×3.5–4.3 μ. The collarettes measure 4.7–7.5×1.8–2.2 μ. The phialospores (Figs. 2, 12) are bacilliform and hyaline, measuring 3.2–10.6×0.7–1.7 μ, and are produced in long persistent chains (Fig. 12) which are held together in a minute drop of slime. In this species no differentiation was evident between the first phialospore and those produced subsequently. Colonies on potato dextrose agar differ from those on malt agar in the greater preponderance of sterile aerial mycelium, which imparts a 'smoke gray' color to the mycelial mat, and in the reduced number of conidiophores produced.

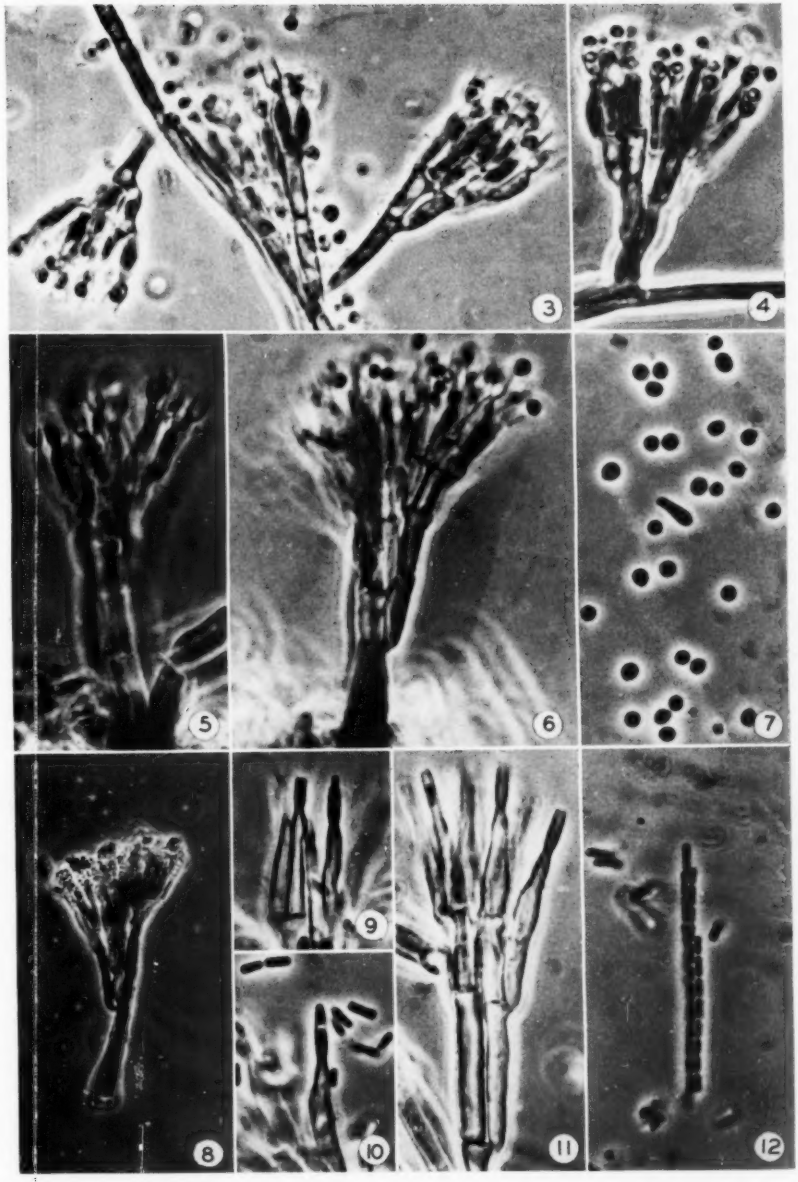
In this paper only two species are placed in the genus *Phialocephala*, but other collections and isolates in Herb. DAOM represent at least eight further undescribed species of *Phialocephala*. However, these are at present represented by single collections only, and it is hoped to publish descriptions of them when additional material becomes available.

FIGS. 3–8. *Phialocephala dimorphospora*. Figs. 3, 4. Conidiophores in culture, ×1000. Figs. 5, 6. Conidiophores from host, ×1000. Fig. 7. Phialospores, ×1000. Fig. 8. Conidiophore from wood, ×400.

FIGS. 9–12. *Phialocephala bactrospora*. Figs. 9, 10. Phialides and phialospores, ×1000. Fig. 11. Sporogenous head, ×1000. Fig. 12. Phialospore chains, ×1000.

All photomicrographs taken under phase contrast.

PLATE I



Kendrick—Can. J. Botany

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References

HUGHES, S. J. 1953. Conidiophores, conidia and classification. Can. J. Botany, **31**, 577–659.

LAGERBERG, T., LUNDBERG, G., and MELIN, E. 1928. Biological and practical researches into blueing in pine and spruce. Svensk Skogsvards. Tidskr. **25**, 145–272.

GROWTH OF CERATOCYSTIS ULMI (BUISMAN) C. MOREAU IN LIQUID CULTURE MEDIA[1]

CAMILIEN GAGNON[2]

Abstract

Ceratocystis ulmi (Buism.) C. Moreau was grown in liquid media with four different nitrogen compounds, eight sugars, and four concentrations of glucose. The fungus was grown at room temperature in the dark as still cultures, and in daylight as still and as shake cultures. The pH of the culture filtrates appeared to be dependent on the sugar used as the main source of carbon. The dry weight of the fungus fluctuated regularly with age. This is shown to be independent of the factors investigated and has been attributed to autolysis of the fungus. Implications of the findings are discussed.

Introduction

Among the many publications on the Dutch elm disease only a few deal with the physiology of the causal fungus *Ceratocystis ulmi* (Buisman) C. Moreau. Schwarz (8) was the first to isolate and demonstrate the pathogenicity of the fungus, which she successfully cultured on a variety of media. Later, more precise information was obtained on the cultural requirements of *C. ulmi*. Ledeboer (4) obtained optimal growth on media containing 5% sucrose or glucose, at a pH between 6 and 7, and at a temperature of 25° C. Tyler and Parker (9) found that *C. ulmi* could grow within a temperature range of 1° to 30° C, the optimal growth being at 27° C. Frederick and Howard (3) stated that the optimal pH for mycelial growth in liquid culture was between 5.2 and 6.5. Zentmeyer *et al.* (10), however, found an optimal pH range of 4.0 to 5.0 with glucose. Burkholder and McVeigh (1) and Robbins and Ma (7) reported that vitamin B_6 (pyridoxine) was required for the growth of *C. ulmi*.

Since the literature on the physiology of *C. ulmi* is somewhat contradictory and incomplete, experiments were carried out to study the effect of various factors on the growth of this fungus in liquid media. The present paper reports on these studies and describes a regular growth fluctuation in the development of the fungus in liquid media.

Materials and Methods

The isolates of *C. ulmi* used in this work were obtained from infected elm trees on the dates and at the localities indicated in Table I.

The media used in these experiments contained: 1.5 g KH_2PO_4, 1.0 g $MgSO_4.7H_2O$, 0.01 g $FeCl_3$, 2.0 g asparagine, 2.0 g yeast extract, 25 g glucose, and distilled water to make 1 l. The pH was adjusted to 5.3 before inoculation. The effect of different nitrogen compounds on the growth of *C. ulmi* was tested by replacing asparagine with equivalent quantities of arginine,

[1]Manuscript received May 18, 1961.
Contribution No. 760 from Forest Entomology and Pathology Branch, Department of Forestry, Ottawa, Canada. Based on part of a thesis submitted to the Faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Master of Science, June, 1960.
[2]Forest Research Laboratory, P. O. Box 35, Sillery, Que., Canada.

TABLE I

Source of culture isolates

Isolate No.	Origin	Date of isolation
M	Montreal, Que.	June 1957
G-453	New York State	Unknown
Q-1	Quebec City, Que.	June 1958
Q-2	Quebec City, Que.	August 1958
N.B.	Woodstock, N. B.	September 1958

NH_4NO_3, or KNO_3. Sucrose, lactose, maltose, glucose, fructose, D-galactose, L-arabinose, and D-xylose were tested by replacing glucose in the medium. Tests with different concentrations of glucose were made at the rates of 5, 10, 20, and 30 g per liter of medium. The tests were carried out with 30 ml of medium in 125-ml Erlenmeyer flasks. The flasks with the fungus were kept in the dark or exposed to daylight. Those in daylight were kept either at rest or agitated on a shaking-machine; those in the dark were at rest.

The dry weight of the cultures was determined daily for the duration of the experiments. Four or five flasks were used each day. Since filter paper proved to be unsatisfactory, Seitz filters (sterilizing type) were used for filtering the cultures. Each filter disk was numbered, placed in a drying-oven at 100° C for 18 to 20 hours, cooled in a desiccator over $CaCl_2$ for about 3 hours, and weighed on a balance having a sensitivity of 0.1 mg. Filtration was performed with the aid of a vacuum pump. The filters with the fungus were then washed with 10 ml of cold distilled water to remove residual soluble materials. These were then oven-dried, cooled, and weighed as before, and the dry weights of the cultures were obtained by difference.

TABLE II

Treatments of cultures of *C. ulmi* in liquid media

Expt. No.	Nutrient	Incubation	Duration (days)	No. at each concn. per day	Total no. of flasks
1	Glucose (4 concns.)	Still culture, daylight	14	5	280
2	Glucose (4 concns.)	Shaken culture, daylight	14	5	280
3	Glucose (3 concns.)	Still culture, darkness	11	4	132
4	Glucose (4 concns.)	Still culture, darkness	14	5	280
5	Glucose (4 concns.)	Shaken culture, daylight	15	5	300
6	8 different sugars	Still culture, daylight	11	4	352
7	8 different sugars	Still culture, darkness	11	4	352
8	4 nitrogen compounds	Still culture, daylight	14	4	224
9	4 nitrogen compounds	Still culture, darkness	14	4	224
10	4 nitrogen compounds	Shaken culture, daylight	15	4	240

Results

The results that follow are based on the experiments listed in Table II.

Although the fungus grew well in still and shaken cultures, the growth was twice as great in the latter (Figs. 1 and 2). For example, the mean dry weight of 6-day-old still cultures was 93 g as compared with 188 g for the mean dry weight of shaken cultures of the same age in medium containing 3.0% glucose.

No differences were obtained in the culture dry weights of isolates Q-1, Q-2, and N.B., when grown either in daylight or in the dark. Also, there was no significant difference between the dry weights of cultures, Q-2 and N.B., when grown in media containing either asparagine, arginine, NH_4NO_3, or KNO_3 as the main source of nitrogen.

Of the eight different sugars tested, the greatest dry-weight (135 g) was obtained with fructose, followed in decreasing order by glucose and L-arab-

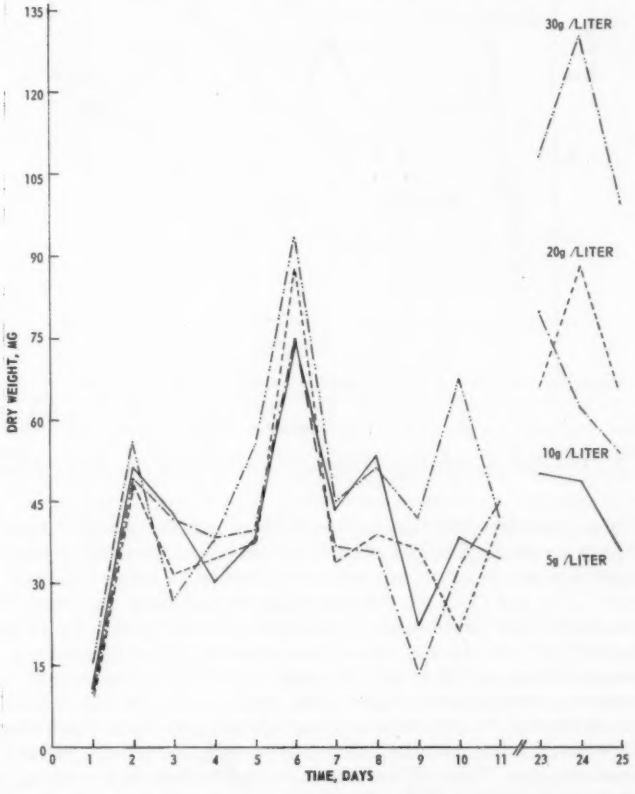


FIG. 1. Dry weights of still liquid cultures containing 5, 10, 20, and 30 g of glucose per liter.

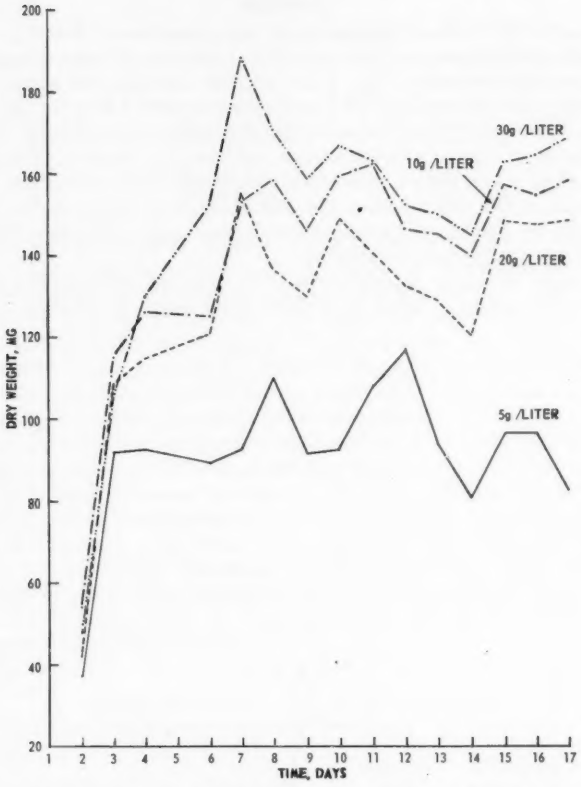


FIG. 2. Dry weights of shaken liquid cultures containing 5, 10, 20, and 30 g of glucose per liter.

inose (110 g), D-xylose (100 g), maltose (90 g), sucrose (85 g), lactose (80 g), and D-galactose (60 g). The pH of the filtrates of cultures grown with glucose, fructose, or sucrose as the main source of carbon decreased during the first week from 5.3 to 4.4 (Fig. 3). This is in agreement with the results of Zentmeyer *et al.* (10) and Feldman *et al.* (2) who reported a decrease in the pH of the cultures of *C. ulmi* to 4.25 after about 1 week of incubation in a medium with glucose. However, this did not occur with the other sugars. When D-xylose was the main carbohydrate of the medium, the pH increased slightly but regularly from 5.3 to 5.9. With maltose the pH increased rapidly during the first 4 days to reach 6.8, and then decreased even more rapidly to 4.4 in 9-day-old culture filtrates. The pH of the culture filtrates with D-galactose was erratic during the first 4 days, then increased markedly, reaching 7.6 after 10 days of growth. In the filtrates of cultures grown with lactose and L-arab-

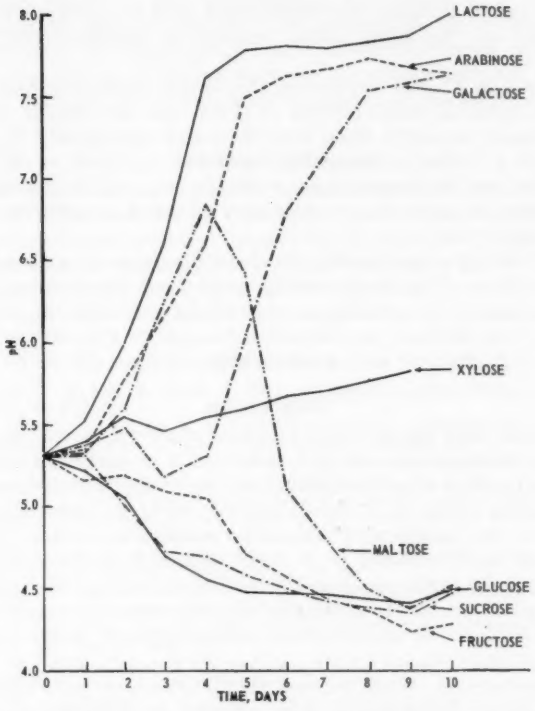


FIG. 3. Variation in pH of filtrates of cultures grown with different sugars.

inose, the pH increased rapidly for the first 5 or 6 days to reach 7.8 and 7.6, respectively; it then remained at about the same level. With lactose, however, a pH of 8.0 was obtained in filtrates of 10-day-old cultures. Because there are two very distinct trends in the pH of the culture filtrates, the indications are that the end-products in the medium differ, and that the fungus probably does not utilize these sugars in the same way. This suggests variations in the carbohydrate metabolism of *C. ulmi*. In the case of D-galactose, it seems that there is a period of adaptation for the fungus.

Isolates M, G-453, and Q-1 were grown as still and shaken cultures in media containing different concentrations of glucose, namely, 5, 10, 20, and 30 g per liter. The increase in the dry weight of the cultures was not necessarily proportional to the increase in the concentrations of glucose in the medium, although, in general, the highest dry weights were produced by those cultures which contained the highest glucose concentrations. In still cultures differences between the dry weights of cultures grown with 5 and 30 g of glucose per liter were statistically significant only after 6 days. The differences were much greater after 24 days (Fig. 1). In shaken cultures a concentration of 10 g/l. of

glucose often gave a higher dry weight than a 20 g/l. concentration; a 5 g/l. concentration, on the other hand, resulted in comparatively less growth (Fig. 2).

Fluctuations in the dry weight of the fungus were observed to occur as the cultures aged. In still cultures (Fig. 1), the dry weight of the fungus increased during the first 2 days, then decreased appreciably during the next 2, followed by a further increase which reached a maximum on the 6th day; this in turn was followed by lower values on the 7th, 8th, and 9th days of incubation. Further successive increases and decreases occurred in cultures incubated for longer periods of time.

In shaken cultures also the same general fluctuations occurred with slight variations in time. The most marked reductions occurred on the 9th and 14th days, at each of the glucose concentrations cited (Fig. 2). Similar growth fluctuations were obtained in cultures incubated in the dark or in daylight, with each of the different sugars and nitrogen compounds tested.

Discussion

The constant and appreciable reduction observed in the dry weights of cultures of *C. ulmi* at intervals of 4 to 6 days is of particular interest. These recurring fluctuations may be explained on the basis of autolysis of the fungus, since degenerating cells have been observed under the microscope liberating protoplasm in the medium. These sharp reductions in dry weight of the fungus cannot be attributed to a sugar depletion of the culture medium, since they occurred at the same age for cultures which had been given different concentrations of glucose. It is possible that this phenomenon is related to the liberation of microendospores observed in this fungus by Ouellette and Gagnon (6).

These observations might explain the fluctuations in the polysaccharide contents in culture filtrates of *C. ulmi* reported by Feldman *et al.* (2). These investigators observed maximal polysaccharide contents of the filtrates on the fourth and seventh days and minimal on the fifth and eighth days, while in the present studies minimal and maximal dry weights of the fungus have been observed after these same periods of incubation (Fig. 1). It seems possible, therefore, that the polysaccharides in the filtrate result from the disintegration of fungal cells by autolysis.

Recent observations by Ouellette (5) and by Ouellette and Gagnon (6) have shown that *C. ulmi* produces microendospores both in culture and within living elm trees. The former investigator has observed empty hyphae and spores in the xylem tissues of this host and has suggested on the basis of these results that autolysis of the fungus might also occur within the tree. Further studies on the factors which may influence this phenomenon are needed to provide a better understanding of its importance in the infection process of the Dutch elm disease.

Résumé

Ce travail résume les résultats d'expériences sur la croissance du *Ceratocystis ulmi* (Buism.) C. Moreau en milieux liquides dont on a varié la composition en utilisant quatre composés d'azote différents, huit sucres, ou du glucose

à diverses concentrations. Ces cultures ont crû à environ 25° C, à l'obscurité et au repos, ou au repos ou agitées à la lumière du jour. Des fluctuations régulières du poids du champignon apparaissent à mesure que la culture vieillit. Ces variations, indépendantes de la composition du milieu, sont attribuées à l'autolyse du champignon. La portée de ces faits est aussi considérée.

Acknowledgments

The author wishes to acknowledge the encouragement and advice received from Dr. D. S. Welch and Dr. M. Gibbs of Cornell University. He also wishes to thank the Department of Agriculture of the Province of Quebec for financial assistance. Further, he is grateful to Dr. René Pomerleau, Dr. D. E. Etheridge, Dr. J. R. Blais, and Dr. G. B. Ouellette of the Forest Research Laboratory, Quebec, for their helpful criticism in the preparation of this paper.

References

1. Burkholder, P. R. and McVeigh, I. 1942. Pyridoxine as a growth factor for Graphium. Science, **95**, 127–128.
2. Feldman, A. W., Caroselli, N. A., and Howard, F. L. 1950. Physiology of toxin production by *Ceratostomella ulmi*. Phytopathology, **40**, 341–354.
3. Frederick, L. and Howard, F. L. 1951. Comparative physiology and pathogenicity of eight isolates of *Ceratostomella ulmi*. Phytopathology, **41**, 12–13.
4. Ledeboer, M. S. J. 1934. Physiologische onderzoekingen over *Ceratostomella ulmi* (Schwarz) Buisman. Thesis, Univ. of Utrecht. Baarn. Rev. Appl. Mycol. **13**, 733–734.
5. Ouellette, G. B. 1960. Studies of host and pathogen in relation to the infection process of the Dutch elm disease caused by *Ceratocystis ulmi* (Buism.) C. Moreau. Ph.D. Thesis, Cornell University, Ithaca, N.Y.
6. Ouellette, G. B. and Gagnon, C. 1960. Formation of microendospores in *Ceratocystis ulmi* (Buism.) C. Moreau. Can. J. Botany, **38**, 235–241.
7. Robbins, W. J. and Ma, R. 1942. Specificity of pyridoxine for *Ceratostomella ulmi*. Torrey Botan. Club Bull. **69**, 342–352.
8. Schwarz, M. B. 1922. The twig wilt and the vascular disease of the elm. Bartlett Research Lab. Bull. **1**, 5–25. *Translated by* Lilian D. Kelsey.
9. Tyler, L. J. and Parker, K. G. 1945. Factors affecting the saprogenic activities of the Dutch elm disease fungus. Phytopathology, **35**, 675–687.
10. Zentmeyer, G. A., Horsfall, J. G., and Wallace, P. P. 1946. Dutch elm disease and its chemotherapy. Conn. Agr. Expt. Sta. Bull. 498.

MICROFUNGI
VII. BRACHYDESMIELLA ARNAUD[1]

S. J. HUGHES

Abstract

Latin diagnoses are provided for the name *Brachydesmiella* Arn. and its type species *B. biseptata* Arn. which is illustrated. The fungus is recorded for the first time from Canada and Britain.

Arnaud (1954, p. 287, Fig. 8*d*) published the generic name *Brachydesmiella* for the single species *B. biseptata* without providing Latin diagnoses for either taxon. He illustrated the fungus and described it as follows: "Conidiophores de 35–45 μ de haut; conidies grosses, en citron; grande cellule médiane noir-opaque et deux cellules terminales incolores; 40×23 μ." The fungus to which Arnaud referred is distinctive and easily recognized from his figure and short description. Although I have not seen Arnaud's collection, which is presumably from France, I consider it justifiable to validate the names by providing Latin diagnoses drawn from collections I have seen from Canada and Britain.

Brachydesmiella Arn. gen. nov.

Fungi imperfecti hyphomycetes.

Conidiophora simplicia, curta, continua vel pauciseptata, hyalina vel pallide brunnea, per proliferationes successivas subapicales elongascentia. Conidiophora proliferationesque poro apicali praedita.

Conidia solitaria, in apicibus conidiophororum proliferationumque oriunda, limoniformia, 2-septata, cellula media magna, crasso-tunicata, atro-brunnea vel atra, cellula terminali, basali quoque, parva, tenuo-tunicata, hyalina vel subhyalina, poro basali praedita.

Brachydesmiella biseptata Arn. sp. nov.

Fungus imperfectus hyphomycetum.

Coloniae atrae, effusae.

Conidiophora dispersa vel plerumque densa, simplicia, erecta, recta vel irregulariter curvata vel geniculata, hyalina vel pallide brunnea, continua vel 3-septata, usque ad 60 μ long., ad basim 3–4 μ lat., apicem versus 6–9 μ lat., in maturitate usque ad 7 cicatricibus brunneis (poris centralibus) praedita.

Conidia solitaria in apicibus conidiophororum proliferationumque successivarum oriunda; plus minusve limoniformia, 2-septata, cellula media magna (circa 30 μ long.), crasso-tunicata, laevia, atro-brunnea vel atra. Cellula terminalis parva (circa 5 μ long.), ad apicem rotundata, subhyalina vel pallide brunnea, tenuo-tunicata, verrucosa, raro projectionibus acutis minutis praedita. Cellula basalis etiam parva (circa 5 μ long.), verrucosa, subhyalina vel pallide brunnea, cicatrice basali plana praedita. Conidia (tota) 36–48×17–21 μ.

[1]Manuscript received April 25, 1961.
Contribution No. 139 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

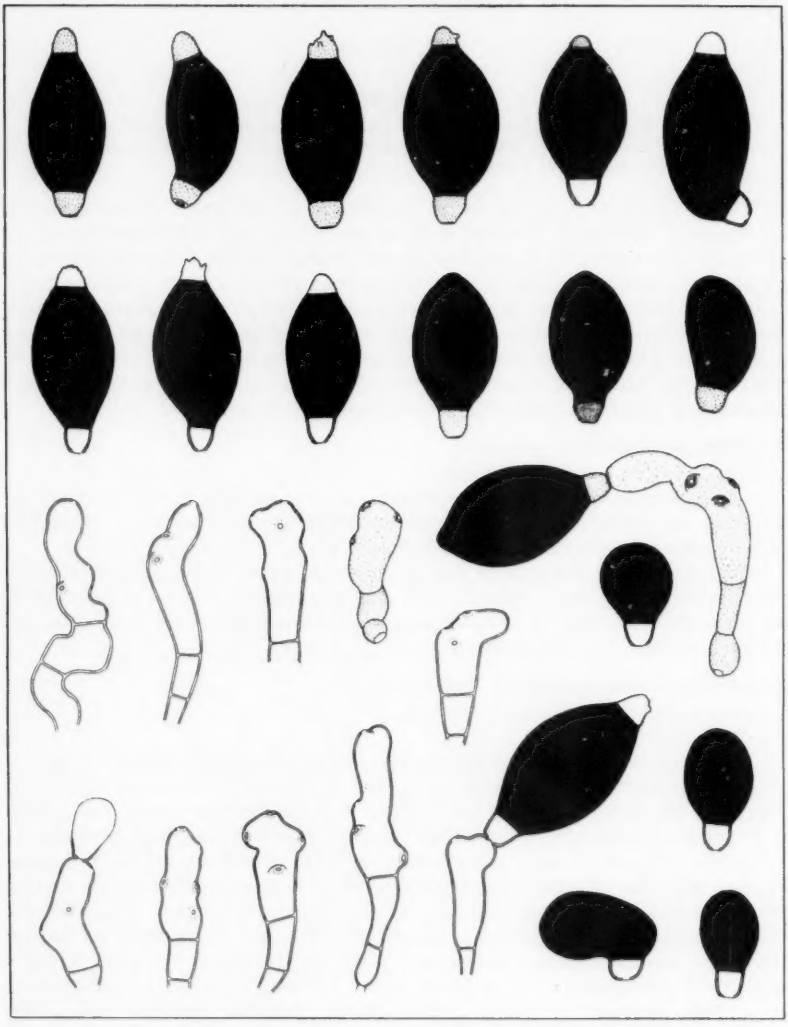


FIG. 1. *Brachydesmiella biseptata*. Conidiophores and conidia from DAOM 44669(b). ×750.

Habitat: in ligno fabrefacto, Kingsmere, Que., Canada, 11.IX.1954, S.J.H., DAOM 44669(*b*), 44663(*a*); in ligno *Fagi sylvatici*, Boxhill, Surrey, Anglia, 1.IX.1946, S.J.H., IMI 6624; in ligno *Fraxini*, Perranzabuloe, Cornwall, Anglia, 14.X.1946, F. Rilstone, IMI 7481.

Typus: G. Arnaud No. 2395.

Brachydesmiella biseptata (Fig. 1) forms shining black colonies when conidiophores and conidia are crowded together.

The conidiophores may be scattered but more usually they are crowded; they are simple, erect, straight or irregularly bent or geniculate, hyaline to pale brown, continuous to 3-septate, up to 60 μ long, 3–4 μ wide at the base and expanding gradually to 6–9 μ wide towards the apex. Conidiophores may bear up to seven circular brown to dark brown conidium scars, each with evidence of a central pore.

Conidia develop singly from a pore at the apex of successively produced growing points. They are more or less oval with apiculate ends, 2-septate, with the central cell very large (about 30 μ long), thick-walled, smooth, dark brown to black. The terminal cell is small (about 5 μ long), rounded, coarsely roughened, and sometimes here and there drawn out into small projections; the basal cell is equally small (about 5 μ long), coarsely roughened, subhyaline to pale brown, and flattened at the basal scar. Conidia measure 36–48$\times$17–21 μ.

Abnormal conidia are not uncommon; these usually lack the small distal cell and are consequently shorter than the 2-septate conidia.

I am obliged to Dr. B. Boivin for correcting the Latin diagnoses.

Reference

ARNAUD, G. 1954. Mycologie concrète: Genera II (suite et fin). Bull. soc. mycol. France, **69**, 265–306.

HETEROPHYLLY IN HIPPURIS, A PROBLEM IN IDENTIFICATION[1]

MARGARET E. MCCULLY[2] AND HUGH M. DALE

Abstract

Although the primordia of all types of leaves in *Hippuris* show no distinguishing characteristics until they are 50 microns long, their further development is strongly influenced by their environment. At maturity they can, for convenience, be thought of as belonging to one of five types, or some intermediate of these types. The five types of leaves are: rhizome, juvenile aquatic, adult aquatic, juvenile aerial, and adult aerial. Field and experimental studies indicate that the leaf form in *Hippuris* is influenced by light and the water relations between the plant and its environment. There is great uniformity of foliar morphology on plants from stocks of diverse origin when grown under uniform conditions, and there is wide diversity of foliar morphology on plants of the same stock material when grown under different environments in the laboratory. Thus the taxonomic subdivision of genus *Hippuris* into species, varieties, and forms using leaf morphology as the main criterion appears questionable.

Introduction

Heterophylly has long interested botanists and has been described in numerous species. Attempts have been made to elucidate the factors controlling the variations in leaf form from node to node on an individual plant. These deal with heteromorphism among mature leaves and have been reviewed (5, 6). One developmental study compares the development of cataphylls and the first foliage leaves of *Viburnum rufidulum* (12). Aquatic plants often show marked heterophylly between aerial and submersed leaves. Recent work has described heterophylly in *Marsilea* species (3, 4), *Callitriche intermedia* (19, 20, 21, 22), *Zizania aquatica* (38), and *Hippuris vulgaris* (29). The ease with which the aquatic environment can be controlled in the laboratory makes water plants particularly useful in experimental work.

Although the primordia of the leaves of *Hippuris* show no distinction until they are 50 μ long, the leaves show a marked morphological plasticity as they mature and can be thought of as aerial, submersed, or rhizome leaves (29). However, these broad categories embrace great variations as seen in Figs. 6 to 15. These variations, combined with the variation in the number of leaves per whorl, have been used as the criteria to divide the genus into three species and/or a number of varieties. The angle at the apex of the leaf and the general shape are thought to be of particular importance in this classification. The purpose of this paper is to discuss the development of leaves in the field and in the laboratory, on apices of clonal material and also from a variety of sources. This study elucidates not only the effect of the environment in modifying the leaf development, but also it suggests the most logical subdivision of the genus *Hippuris* in the light of these findings.

[1]Manuscript received April 13, 1961.
Contribution from Department of Botany, Ontario Agricultural College, Guelph, Ontario.
[2]Present address: St. Felix School, Southwold, Suffolk, England.

Leaf Growth

Development of Submersed Leaves

The primordia of leaves in *Hippuris* are not initiated at a constant distance from the tip of the apex. The distance from the tip is usually greater in apical thimbles grown under water than in the air. However, the initiation and growth until they are 50 μ long is identical. The first signs of initiation are periclinal divisions in the second layer of cells. Usually each primordium results from the division and growth of seven cells situated in three horizontal rows (2, 3, and 2 cells), and the concurrent expansion of the surface layer of the cells. As it pushes out from the surface of the apical cone the vertical mid-section of each primordium consists of five vertical layers of cells: the upper and lower superficial layers and three middle layers (29). Towards the tip of each growing leaf there may be a single middle layer and this may be absent at the extreme tip.

The following will describe the development of these primordia into submersed leaves of the type we have found in fresh-water habitats in Southern Ontario (south of 46° N.). These leaves when mature are long and flaccid. Plants bearing only this type of leaf have been classified as *Hippuris vulgaris* forma *fluviatilis* (Cass. and Germ.) Gluck. A specimen of a plant bearing this type of leaf is illustrated in Fig. 6. The discussion of other submersed leaves will be dealt with subsequently.

Elongation of the young primordium occurs principally by apical growth. The mesophyll of aquatic leaves extends as a result of anticlinal divisions of a single subapical cell and its derivatives. This growth is accompanied by cell division and enlargement in the epidermal layers. Young leaves about 1 mm long have the distal quarter of their length composed of an upper and a lower epidermis and a single mesophyll layer. The cells of the central portion of the mesophyll along the leaf axis form a two- or three-layered strand. Further extension of the leaf occurs by cell enlargement and intercalary cell division.

Lateral expansion of the primordium is concurrent with its elongation. Increase in width is produced by cell enlargement and by anticlinal divisions in the two strips of cells along the margins of the leaf. The first one or two cells and the next three to five cells produced by these submarginal initials divide periclinally to produce respectively two and three layers of mesophyll on each side of the leaf axis. Beyond this relatively narrow strip no periclinal divisions occur, and each row of submarginal initials produces a broad band of lamina composed of a single mesophyll layer. Anticlinal divisions in the upper and lower epidermal cells keep pace with the increasing width of the leaf blade. Cell division in the epidermis continues after the cessation of submarginal meristem activity so that a narrow strip composed only of two layers of epidermal cells is produced around the margin of the mature leaf.

When a primordium is 50 μ long a single row of cells in the center of the mesophyll along the leaf axis differentiates acropetally into procambium. This differentiation continues as the primordium increases in length. When it is approximately 600 μ long, xylem vessels begin forming relatively slowly, in a basifugal direction.

Glandular trichomes develop from cells of both the upper and the lower epidermis. Each appears initially as a bulge on the surface of a short cell

when the young leaves are approximately 600 μ long. Following its enlargement this cell divides in the same plane. The lower cells form a foot and the outermost one undergoes anticlinal division to produce a cap composed of 12 to 25 wedge-shaped secretory cells. These cells are deep-staining and their thickened walls have thin rodlike protrusions. The development of trichomes in *Hippuris* is similar to that described for *Ligustrum* (15).

At maturity aquatic leaves have a single mid-vein and no laterals (Fig. 1*a*). This vein terminates at the leaf tip in a hydathode which may or may not contain a pore which resembles a large stoma, but appears to lack the ability to close. No normal stomata are produced on either epidermis of an aquatic leaf.

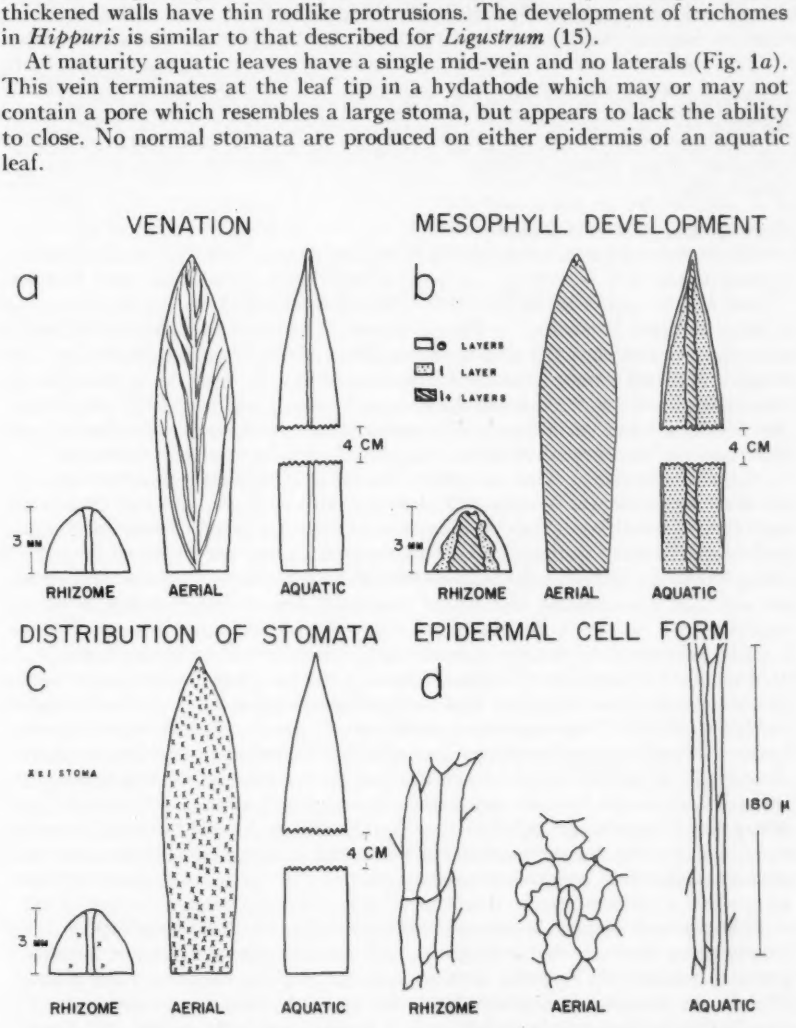


FIG. 1. Drawings of typical leaves representing three types: rhizome, adult aerial, and adult aquatic. Comparison can be made for *a*, venation; *b*, mesophyll development; *c*, the distribution of stomata; and *d*, the form of the epidermal cells.

Most of the lamina of a mature leaf has only one layer of mesophyll cells (Fig. 1*b*). There is a relatively narrow band on each side of the leaf axis containing two or three mesophyll layers, and two narrow strips along each leaf margin composed of only the upper and lower epidermis. The small amount of mesophyll, and the lack of development of a palisade layer, as well as the weak vascularization, result in the very flexible and flaccid nature of these leaves.

The epidermal cells of mature aquatic leaves are very characteristic, being long and narrow with straight cell walls (Fig. 1*d*). These walls are thin and only slightly cutinized. Contrary to the observations of Costantine (9) and Chatin (7), no chloroplasts were seen either in upper or in lower epidermal cells. Large droplets which retained Sudan IV stain were often observed, however, in these cells.

Development of Aerial Leaves

When the apex of a stem of *Hippuris* growing in Ontario south of 46° N. grows in the air, leaves of a type different from those described develop. These will be called aerial leaves in this discussion and can be considered as similar to those illustrated in Figs. 7 and 11. There is no anatomical difference between primordia of the two types of apices before they are 50 μ long. This stage is reached sooner in aerial stems than in aquatic ones; at approximately the sixth plastochron below the apex in the former, and the eighth in the latter. At this length the aerial primordia become more rounded than the aquatic ones because the mesophyll at the tip increases to two cells in thickness.

Considerable elongation of aerial leaves is produced by apical growth. At least two cells act as subapical initials. Anticlinal divisions of these cells, and their derivatives, produce extension of the mesophyll. Subsequent early periclinal divisions in the derivatives typically form six layers of mesophyll along the leaf axis. In aerial leaves of 1 mm length, these layers extend almost to the leaf tip, making the leaves unmistakably distinguishable from the aquatic ones, in which the distal portion contains only one mesophyll layer. Lateral growth of the lamina is produced by marginal and submarginal initials. Unlike the development of aquatic leaves, however, periclinal divisions in the derivatives produce up to four layers of mesophyll adjacent to the leaf margins.

Differentiation of procambium occurs when the young leaves are approximately 50 μ long and proceeds faster than in aquatic primordia previously described. Xylem is formed when the leaf is approximately 600 μ long. Its differentiation also is more rapid than in aquatic leaves. Some primordia of 600 μ have vessels throughout their complete length, while others from the same whorl show no vascularization. No aerial leaves were seen where xylem differentiation had proceeded for only part of their length. It was, therefore, impossible to determine the direction of this differentiation.

Palisade and spongy layers are distinguishable in the mesophyll of aerial leaves when they are 600 μ long. Aquatic leaves, however, do not develop a palisade mesophyll. Stomata first appear on aerial leaves at the 600 μ stage. They form in equal numbers on both the upper and the lower epidermis. The development of glandular trichomes is similar on both aerial and aquatic leaves. They are, however, less numerous on the former and do not appear until these leaves are at least 600 μ long.

Lateral veins develop in the aerial leaves when they are approximately 2 mm long. Typically five or more of these are produced on each side of the main vascular strand. At maturity the laminae of aerial leaves contain up to six layers of mesophyll (Fig. 1*b*) which is clearly differentiated into spongy and palisade areas. The epidermal cells (Fig. 1*d*) are short and broad, with sinuate lateral walls, and thick outer walls which are relatively heavily cutinized.

Development of Rhizome Leaves

The initiation and early development of the rhizome leaves is similar to that of the aquatic and aerial ones. However, insufficient suitable sections were available to determine this conclusively. At maturity these leaves are quite distinctive. As shown in Fig. 1, they have no lateral vein development and the number of layers of mesophyll varies in different areas of the lamina. A few stomata are usually present and the epidermal cells are broadly rectangular in outline, with straight lateral walls. Little cuticle is formed on rhizome leaves.

Modification of Growth Pattern

Variations in Leaf Form on Individual Branches

In Ontario south of 46° N., a *Hippuris* stem, which is rooted underwater but growing through the surface, normally produces a distinctly different leaf type in each environment. Transitional forms between the typical aerial and aquatic leaves may also develop near the interface between the two media. This, however, is not the only heterophylly which occurs in *Hippuris* plants. A gradual change in leaf form can be seen even on branches that are entirely aerial or aquatic. Figures 2 and 3 show the gradations in shape, venation, mesophyll development, distribution of stomata, and epidermal cell form in leaves from the lower to the upper verticils of two branches—one which has developed entirely in the air as a lateral shoot, and the other which has always been completely submersed. In these submersed leaves only a single midvein occurs.

Goebel describes heteromorphism in many plants and suggests that the leaves of the lower nodes are juvenile forms which have been arrested at various stages in their development toward the adult type. He terms this production of adult and juvenile forms "heteroblastic development" (16). If the leaves of the basal whorl of both aerial and aquatic branches are considered to be the most juvenile, it can be postulated that four basic leaf types are produced by upright *Hippuris* stems—juvenile and adult aquatic leaves, and juvenile and adult aerial leaves. A fifth type of leaf is the rhizome type. All other leaf forms observed can be thought of as variations of these basic types produced by the interaction of the environment under which they developed, and their physiological age.

Goebel considers that the dimorphism between aerial and submersed leaves, shown by many aquatics, is actually heteroblastic development, with aquatic leaves being merely juvenile aerial ones (16). This hypothesis is refuted by Allsopp, who concludes from experimental work that heteroblastic development and the production of land and water forms in the amphibious fern *Marsilea* are two discrete processes (4). This present study with *Hippuris* supports the latter view.

Variation of Hippuris Material in the Field

A sharp demarcation in leaf form usually exists when a stem emerges from the water. When growing in deep water (up to 6 ft) stems often remain completely submersed having short internodes and producing only the long flaccid leaves. At the other extreme when plants are growing in exposed mud or covered by only an inch or less of water the aerial type of leaf is usually produced along the full length of the stem. At the outlet of Rodger's Creek, which flows into South Bay, Manitoulin Island (45° 36′ N., 81° 56′ W.), *Hippuris* grows in abundance. The level of this peaty stream, which discharges into the waters of Lake Huron, had risen a week prior to a visit in July 1957, submerging a great many stems that had been in the air. Close to their apices these stems clearly showed an upward sequence of long flaccid leaves, then short stiff leaves and followed by long flaccid ones. The leaves, however, were often atypical of either the aerial or aquatic form. Although long and flaccid, at least some of the leaves in all the upper whorls examined had one or two

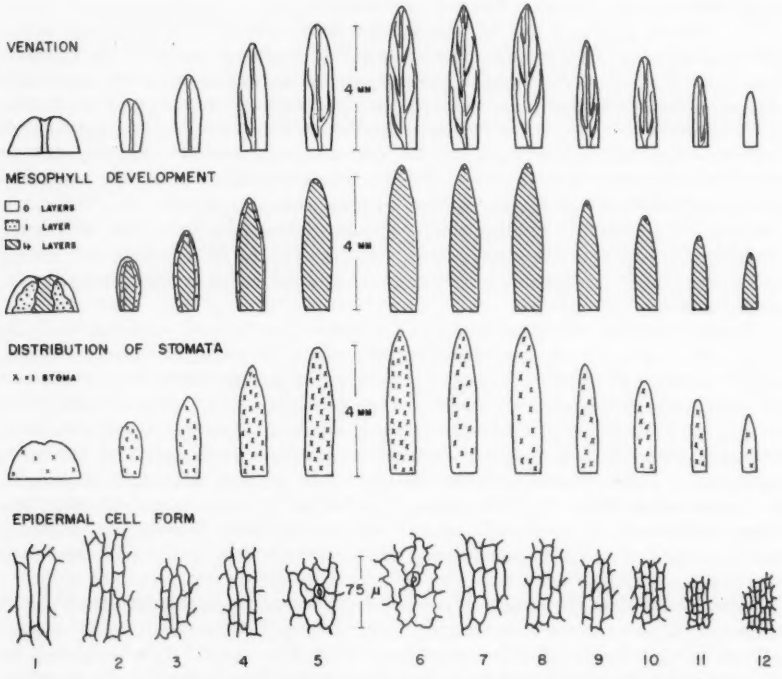


FIG. 2. Drawings of representative leaves from a lateral branch of *Hippuris*. This branch developed entirely in the air. The leaves are from successive whorls with the oldest, first-developed one on the left and the youngest, newly-developed one on the right. The features represented are those in Fig. 1.

pairs of weakly developed lateral veins at their distal ends, and all of them had shortened epidermal cells, thicker mesophyll, and some stomata in the same region. This seems to indicate that lateral vein development occurs somewhat later than that of other characteristics mentioned.

In the same area a great many *Hippuris* stems were seen along the turfy banks of the stream. These were particularly small and dark green in color on the bank facing south. The rhizomes of the material growing in this site were traced. There was a marked uniformity of these underground parts that formed an extensive network under both the stream and the banks. There was no indication that they were not of the same stock.

Some *Hippuris* plants grow 80 miles south of Manitoulin Island at Owen Sound (44° 35′ N., 80° 55′ W.) in turbulent water. At the beginning of August 1959 many long stems had produced aerial-type leaves on branches carried horizontally under the water by the current. These branches often produced aerial-type leaves for more than a foot before the tips suddenly turned to grow vertically into the air. These underwater aerial leaves were similar to those formed in the air, with respect to venation, stomates, mesophyll, and epidermal development, but were shorter, broader, and tended to be rounded at the tip.

Leafy aquatic stems from the same stand at Owen Sound occasionally showed extensive branching. This type of growth was not seen in material from any other location. Since the establishment of this stand in fast-running relatively shallow water was unique among those seen, it appears that the branching may result from the snapping off of the shoot apices by the current. Small lateral branches were occasionally seen on aerial stems from which the apices had been cropped by waterfowl. The inhibition of lateral growth by the apical meristem was demonstrated in the laboratory. If stem sections bearing four or five nodes but no apex were tied to a glass rod and submerged

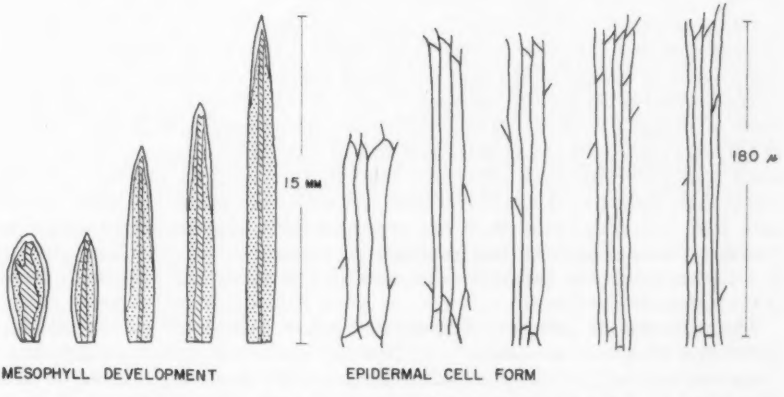


FIG. 3. Drawing as in Fig. 2 of leaves from successive whorls developed entirely underwater. These leaves all lack stomata, and have only a single mid-vein. Mesophyll development and epidermal cell form are represented.

in water, one or two lateral branches were usually developed. If, however, an apical stem section was used, growth occurred only at the tip, and no lateral shoots were produced. Frequently branches, regardless of the stimulating factors which induced their formation, began their growth with two or three nodes of rhizomelike leaves (i.e. very short and broad) separated by long internodes.

Interesting variations were seen in the angle of the leaves relative to the stem. On both aerial and submersed branches the positions of the leaves ranged from upcurving to strongly recurving, both extremes sometimes occurring on an individual stem. There appeared to be a tendency toward an increasing downward slope of the leaves with increasing light. This was emphasized by the appearance of the *Hippuris* plants in the Montreal Botanical Gardens. Here, aerial stems which were growing in mud in an aquarium exposed to full sunlight had leaves so recurved that the adaxial angle was approximately 155°.

Stands visited in more northern areas show that not all submersed material is of the same pattern. At Great Whale River (55° 17′ N., 77° 47′ W.) and Fort Chimo (58° 09′ N., 68° 18′ W.) in deeper water typical long, flaccid aquatic leaves were borne on stems with short internodes (Fig. 6). In the shallower permanent pools of 12 to 18 in. depth, the submersed leaves were of the same pattern as the aerial leaves although with slightly broader lamina than those of the typical aerial type. The stems had long internodes and were the firm emergent form, illustrated by the long branch at the left of Fig. 16. The stems and leaves at water level were strongly colored red, probably owing to anthocyanin pigments. These plants were coarser, with thicker stems and broader, shorter leaves than those in Southern Ontario. Further there were fewer leaves per whorl and they tended to be more obtuse similar to those at the tips of Fig. 9. In other stands visited in the Northwest Territories this pattern was similar although at Cambridge Bay (69° 07′ N., 104° 47′ W.) and Coral Harbour (64° 09′ N., 83° 05′ W.) some of the submersed material in about 2 ft of water had flaccid leaves of only one-half the length of similar material farther south. These stems, however, were growing in the same pools as others which had developed aerial-type leaves from the ooze at the bottom of the pool to the apex. Figure 13 is a photograph of a herbarium sheet of similar flaccid-leafed material collected in Lapland. Some variation in the aerial-type leaves and growth pattern was also noted. Lateral vein development was generally more extensive than in the southern plants, although a specimen collected at Coral Harbour had only one pair at the basal end of each leaf, including those from the upper verticils. Further study would be necessary to establish whether flooding with salt water from the Arctic Ocean is a factor producing the variations seen in the pattern of growth in those pools closest to the shore.

The existence of two very dissimilar types of submersed shoots growing sometimes within a few inches of each other in the same pool suggests that these might belong to different subdivisions of the genus and should be distinguished as different varieties or ecotypes (14, 18, 25, 35). However, as is demonstrated below, this is not the case.

Experimental Production of Morphological Variations

Hippuris is easily propagated from stem cuttings or bud-bearing rhizome sections. The latter method is particularly useful as such sections can be obtained even by fishing under the ice in the winter for the perennial rhizomes. Plants were grown under different conditions in the laboratory to determine the morphological effects of various environments.

Production of Aerial and Aquatic Shoots

Stock material was collected in August 1959 at Great Whale River, Frobisher Bay, Coral Harbour, and Fort Chimo in the Canadian Arctic. This was grown, along with plants from various stands in Southern Ontario, under uniform conditions in the laboratory. As previously mentioned the Arctic material was quite dissimilar morphologically to that collected farther south. Cuttings were rooted in small flowerpots containing a good-quality loam, covered by 0.5 in. of fine sand. Single uniform lots of loam and sand were used throughout the present experimental work. The pots were kept saturated by being set in a tray of distilled water, and were placed in a broad-spectrum light panel (30), under an intensity of 1000 foot-candles. There was no significant difference in the morphology of plants from the various stock sources grown under these conditions. Only aerial-type shoots were produced. The leaves were 6 to 10 mm long, approximately 1 mm wide, and in whorls of five to nine. These results were suprisingly uniform considering the heteromorphism of the stock material used.

Hippuris cuttings from the various locations were also grown in an aquatic environment in the laboratory. Rhizome and leafy stem sections were planted in 3.5-liter wide-mouthed glass jars, containing 2 in. of loam covered by 0.5 in. of sand. The jars were filled with distilled water so that the young plants were submerged about 6 in. They were placed in the same light panel as the pots mentioned above, and the water level was kept constant by the addition of distilled water as necessary. In every case while submerged these plants produced only the thin, flexible aquatic leaves which completely lacked stomata or lateral veins (Fig. 14 illustrates this leaf type). After reaching the water surface, most apices abruptly began producing aerial leaves of the type illustrated in Fig. 11, but some of those close to the edges of the jar tended to cling to the glass surface, with the long aquatic leaves tightly enclosing the apex (as in Fig. 13). The latter stems often grew as much as 1-1/2 in. above the water surface before suddenly shifting to the production of aerial leaves. No indication of intermediate leaf types was seen under these conditions.

Production of Transitional Leaf Forms

Small pots containing typical aerial shoots which had been grown in 1000 ft-c of light for 1-1/2 months were submerged in 3.5-liter wide-mouthed glass jars containing sufficient distilled water so that the tallest shoot was covered by at least 0.5 in. The water in the jars was changed every 2 days. After 10 days all the shoots over 4 cm long had died, but the shorter ones were healthy, and were producing long flexible, typically submersed leaves at their apices. The original mature leaves, however, retained their aerial character. These branches were fixed in formalin–aceto-alcohol and whole mounts of the leaves

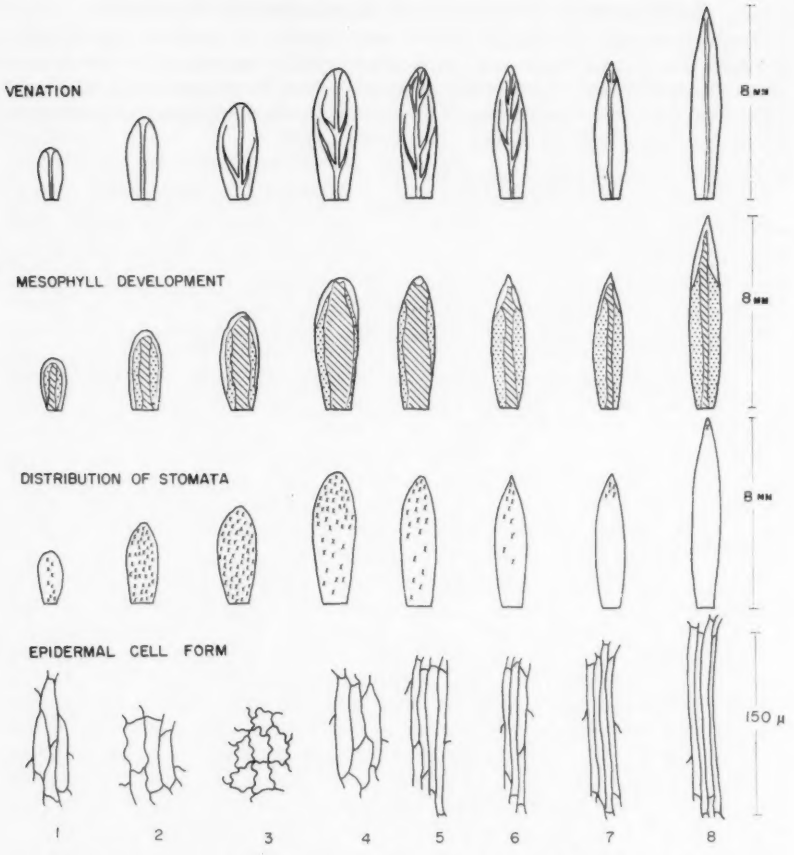


FIG. 4. Drawings as in Fig. 2 of leaves from successive whorls on a branch initially in the air, but transferred to water.

examined microscopically. Typical leaf morphology observed from the lowest to the upper nodes is shown in Fig. 4. It is evident that a definite gradation exists in leaf shape, stoma production, epidermal cell structure, and mesophyll and lateral vein development.

Costantine (10) also submerged aerial *Hippuris* branches. He observed a shift to the production of the aquatic leaf form but does not report the development of any transitional types.

Growth under a Continuous Mist

Small pots of well-established *Hippuris* plants producing typical aerial-type shoots were placed under a continuous mist of tap water in a light panel,

under a 16-hour-day photoperiod. No measurement was made of the light received by the plants under the mist, but the intensity at the level of the pots was 1000 ft-c, in the absence of the spray. After 1 week some of the original shoots over 6 in. high were dead, but the remaining long ones and most of the short ones continued to develop aerial leaves. A few of the short stems under 1 in., and approximately one-half of all the new shoots produced entirely under the mist, began forming leaves identical with those normally developed underwater. These aquatic shoots, although more flexible than aerial ones, remained upright often to a height of 6 to 8 in. The long leaves all were upturned toward the apex and clung closely to the stem. The upright shoot and part of the decumbent shoot at the right of the pot illustrated in Fig. 16 demonstrate this. The remainder of the young shoots grew upright very quickly, producing long internodes and leaves which were similar to the aerial type but shorter, broader, and slightly rounded at the tips. These stems were quite stiff and remained upright longer than either the original aerial branches or the aquatic ones and are illustrated at the left of Fig. 16.

After 6 weeks in the mist environment many of the stems showed shifts from one leaf type to the other. Figure 5 shows a branch which has aquatic leaves on the basal portion, rounded aerial leaves in the mid-section, and aquatic leaves at the apex. Transitional leaf types were often observed on such branches, the most usual variation being long, thin, flaccid, aquatic leaves with some stoma and lateral vein development.

This experiment was repeated using a light intensity of 400 ft-c. Under these conditions there was a greater tendency toward the development of aquatic leaves, with most of the young branches, and even some of the original tall aerial shoots, producing this type.

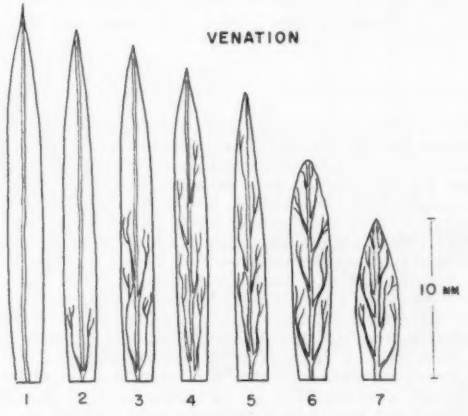


FIG. 5. Drawings as in Fig. 2 of leaves from successive whorls on a branch initially submersed in water. The upper part of this branch emerged from the water. Venation only is shown.

Growth in Saline Solutions

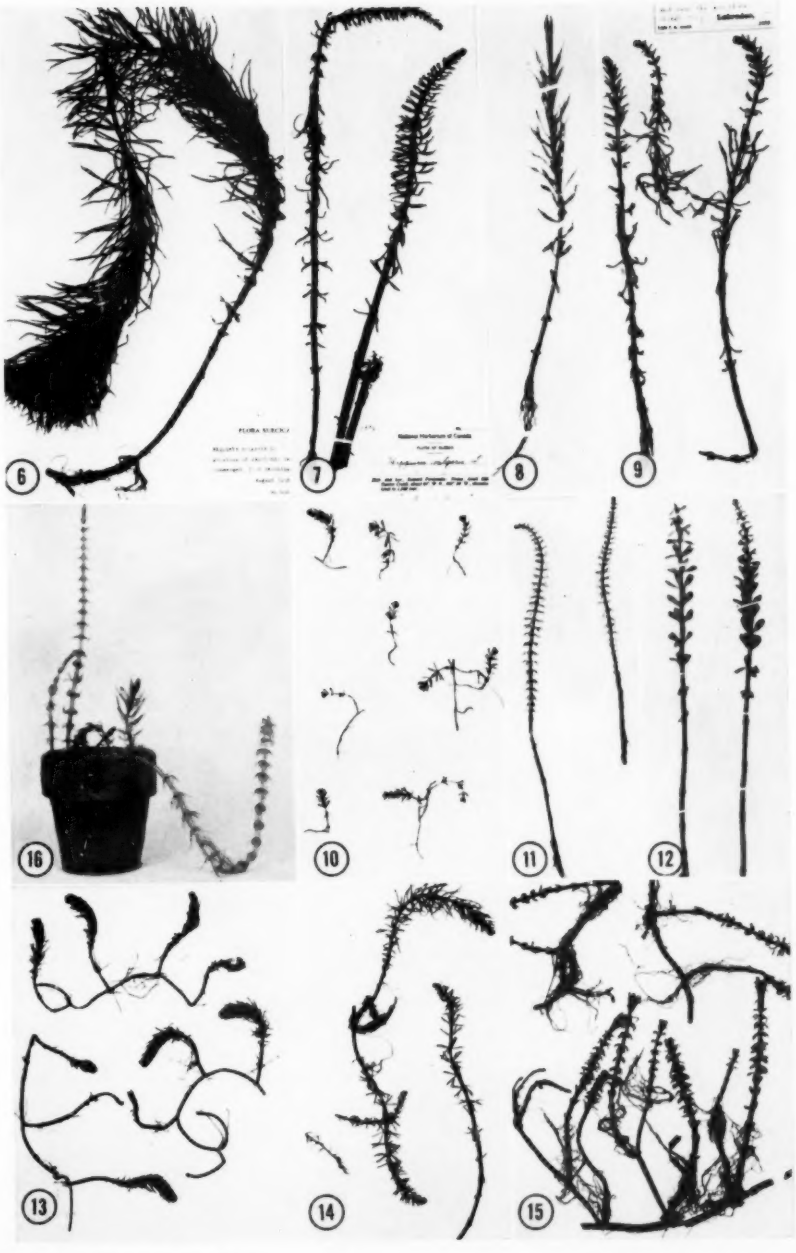
The morphological effects of growing plants in solutions of various osmotic pressures have been demonstrated with *Veronica anagallis* (8), with *Marsilea* species (34), and with *Callitriche intermedia* (19, 21). These plants are either aquatic or amphibious, and show marked heterophylly between their aerial and water leaves. In the experiments they were grown in solutions of high osmotic concentration—*Veronica* in 10% glucose, *Marsilea* in various solutions of sugars and inorganic salts, and *Callitriche* in 30% sea water. In each case leaves were developed which resembled those normally produced in an aerial environment.

Rhizome sections of *Hippuris* bearing terminal and lateral buds were grown in tap water, and in solutions of 10% and 30% artificial sea water. The latter were made using Brujewicz's formula (34). The cuttings were tied to pieces of glass rod and submerged in wide-mouthed 3.5-liter glass jars containing 4 in. of the solution. These were placed in a greenhouse and aerated continuously. Because of the relatively slow development of *Hippuris* it was difficult at first to keep the young shoots free of bacterial contamination until sufficient new leaf whorls had been produced. This problem was overcome, however, by changing the solution every 2 days and keeping the jars in a constant-temperature tank at 12° C. The forms of these shoots resemble those in Fig. 15.

The cuttings in both tap water and 10% sea water produced morphologically normal aquatic leaves. The ones in 30% sea water, however, developed obovate–obtuse leaves approximately 4 mm long and 3 mm wide, in whorls of four to six. The epidermal cells of these leaves were rectangular in outline, with sinuate lateral walls. There were some stomata developed on both surfaces and the laminae contained two or more mesophyll layers over their entire area. Except for the complete absence of lateral veins in these leaves, they were similar in form to the aerial type seen on the *Hippuris* plants collected in the Arctic.

FIGS. 6–15. Photographs of herbarium sheets of *Hippuris*. ×1/6. These illustrate some of the variations seen in this genus. The following data are from these sheets. Fig. 6. *H. vulgaris* L., Sweden, Province of Jamtland, Lake Råtanssjön, submerged, just reaching the surface, August 12, 1960, Leg. Gunnar Lohammar. Fig. 7. *H. vulgaris* L., Alaska, shallow tundra lake, August 6–10, 1926, Coll. A. E. and R. T. Porsild. Fig. 8. *H. tetraphylla* L. f. forma *lacunarum* Dutilly and Lepage, James Bay, holotype, saline water of river mouth, Sept. 9, 1952, Coll. A. Dutilly, E. Lepage, and M. Duman. Fig. 9. *H. maritima* Hell., Labrador, Middle Bay, pool near the seashore, 1882, Leg. J. A. Allen. Fig. 10. *H. montana* Ledeb., Alaska, Aleutian Islands, Unalaska, August 14, 1932, Leg. W. J. Eyerdam. Fig. 11. *H. vulgaris* L., Terrae Novae (Canada), Humber District, White Lake (south of), in shallow pool in wet, open peaty place, July 23, 1951, Leg. E. Rouleau. Fig. 12. *H. tetraphylla* L. f. Alaska, Norton Sound, Unalaklet brackish lagoon, July 29–30, 1926, A. E. and R. T. Porsild. Fig. 13. *H. vulgaris* L., Sweden, Province of Lapland, Lycksele Lappmark, Lake Stora Umevattnet, submerged in water of about 1 meter depth, August 16, 1960, Leg. Gunnar Lohammar. Fig. 14. *H. vulgaris* L., Arctic Alaska, 0.5 mi. south of Colville River, in both fast and pond water along creek, August 10, 1952, Coll. G. H. Ward. Fig. 15. *H. vulgaris* L., Aleutian Islands, Massacre Bay, Alexai Pass, in stagnant pool, at 900 ft elev., July 7, 1945, Lt. G. B. Van Schaack Coll.

FIG. 16. Plant grown under continuous mist, ×1/6. The longer shoots were all produced after the pot was placed in this environment.



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Investigation of the Effects of Various Oxygen Tensions on Morphology

Leafy stem and rhizome cuttings of *Hippuris* were grown in 3.5-liter wide-mouthed glass jars containing 1.5 in. of loam covered by 0.5 in. of sand and 7 in. of distilled water. The jars were placed on a table 5 ft from northeast windows. Fluorescent lights provided an additional 200 ft-c of light and were used to extend the daily photoperiod to 16 hours. One lot of jars was used as a control; the second was subjected to continuous bubbling with a mixture of 99% oxygen and 1% carbon dioxide; the third to 50% oxygen, 49% nitrogen, and 1% carbon dioxide; and the fourth to 20% oxygen, 79% nitrogen, and 1% carbon dioxide. By using fritted-glass gas-dispersion tubes connected to a needle valve on the cylinder of gas mixture, uniform streams of small bubbles were introduced into each jar. After 2 months no significant morphological differences could be seen between the plants of the control and those growing under the three treatments. In every case the branch apices only developed aerial leaves (as in Fig. 11) when they were above water and aquatic ones (as in Fig. 13 or 14) when submersed or when emerged but adhering to the surface of the jar.

Discussion

Disagreement exists as to when heteromorphism is first apparent in developing leaves. Cross does not report any changes in the apices of *Viburnum rufidulum* when they are producing cataphylls or foliage leaves, and finds the primordia similar until they are 500 μ long (12). McCallum concludes that the development of very young aquatic and aerial leaves of *Proserpinaca palustris* is similar, as the primordia can change from one type to the other when as much as 4 mm long (27). Later investigations demonstrate that, in *Zea mays* and *Marsilea* at least, differences in leaf form are correlated with changes in size and shape of the stem apex (1, 3).

In *Hippuris* foliar heteromorphism is accompanied by variations in shape and size of the shoot apices although the primordia of the different leaf types are similar morphologically until they are 50 μ long (29). The environmental influence which is apparent at this very early stage, however, is not irreversible as can be seen by the relatively rapid development of aquatic leaves when aerial shoots are submerged. The transitional leaf forms which have been described indicate that the shift in *Hippuris* is not necessarily as abrupt as that between the land and water forms in *Marsilea* (3). These intermediate types also show that foliar morphological characters may be independently affected by the environment and, as a result, develop to different degrees in the same leaf. Thus in Fig. 4 epidermal cell shape and leaf shape appear to be controlled separately from each other, and from mesophyll, stoma, and lateral vein development. The development of these latter three is affected by the environment at approximately the same stage in primordial growth, whereas leaf shape is determined relatively earlier, and epidermal cell form later.

Sections made of developing leaves show that vascularization of the main vein occurs acropetally. In the normal sequence from juvenile to adult leaves (Fig. 2) it appears that lateral veins also develop from the base toward the leaf tip. This is evident as well in the underdeveloped leaf No. 3 (Fig. 4)

which has only matured enough so that the lowest pair of laterals has formed. When aerial stems are submerged the leaves developing from the younger primordia fail to produce lateral veins, first at their basal ends, then progressively toward their vertices at successively higher nodes. In every case in the present experimental work, when young aerial shoots were immersed, one or two whorls developed leaves similarly to leaf No. 7 (Fig. 4), where a single pair of laterals occurs at the distal end. The venation of these transitional leaves seems to indicate not acropetal, but basipetal development. This apparent incongruity can only be resolved by considering that two separate stages are involved in the formation of lateral veins. The initial process occurs in a basipetal direction and will determine whether lateral veins may develop or not. This process is irreversible. If the aerial pattern is established, the second stage, that of lateral vein differentiation, then proceeds in an acropetal direction. Thus the venation of leaf No. 7 (Fig. 4) may have resulted from the stem being submerged at a time when the primordium was small enough so that the aerial character had been impressed on its distal end alone. The remainder of the young leaf then developed underwater with the result that the lateral veins only formed near the tip.

Transitional leaves observed at Owen Sound (Fig. 5) show progressively more lateral venation toward their vertices, at successively higher nodes. Such development suggests a greater exposure of the upper primordia to the air. For varying distances from the tip, dependent upon their age, these primordia have developed the aquatic character which prevents lateral vein formation. When exposed to air, lateral veins could only be differentiated in the part of the leaf where the influence of the aquatic environment had not inhibited their development.

The research into factors causing heterophylly between aerial and submersed portions of water plants has been reviewed (4, 5, 6, 20, 21, 22). Such changes in leaf morphology have been attributed to various factors, which mostly fall into two general groups—differences in the nutritive level of the plants or variations in the water relations within the cells.

The production of leaves resembling aerial ones by *Hippuris* cuttings growing in a 30% solution of artificial sea water, and the development of aquatic leaves by plants growing in a saturated atmosphere, indicate that the water relations between the cells of the apex and the surrounding medium do influence the type of leaf which is formed.

The results obtained by growing *Hippuris* plants under a continuous mist at different light levels show that the leaf form is also in some way influenced by light. Apparently in the saturated environment the osmotic concentration within the cells was approaching the critical level for the production of the two leaf types. At this point a light intensity of 1000 ft-c was sufficient to cause many of the branches to develop broad, rounded aerial-type leaves whereas at one-half this illumination mostly aquatic leaves were produced.

Two possible means by which cell osmotic concentration and light could control foliar morphology have been postulated. Jones suggests that the leaf form in *Callitriche intermedia* may be influenced by cell turgor pressure, through its control of auxin production and translocation (21). Both the

osmotic concentration of the tissues and light can affect mesophyll development in *Hedera helix* by changing the starch–sugar ratio in the cells. A high sugar content resulting in palisade development is produced by partial desiccation of the leaves. This can occur even in a saturated atmosphere under high light intensity. With less light, a humid environment results in a high starch content and spongy mesophyll formation only (37). The concentration of oxygen even in well-aerated water is very low compared with that in air. In the present study this might produce the marked heterophylly between emerged and submersed *Hippuris* branches. The formation of aquatic leaves by plants in water through which 99% oxygen was bubbled continuously, as well as by those exposed to a relatively high oxygen availability under a continuous mist, shows however that no apparent relationship exists between leaf morphology in *Hippuris* and the oxygen tension of the medium. These results are not in agreement with those from experiments with *Elodea*, where shorter leaves and stem internodes were produced with increased oxygen and reduced carbon dioxide tension (13).

The short, broad, aerial leaves which were produced by *Hippuris* stems growing in a saturated atmosphere closely resembled those on material collected in the shallow Arctic pools where these leaves were frequently developed even on submersed stems. Seidelin reports that this leaf type always occurs on branches growing underwater in Denmark (33). Costantine found that submersed branches developed long, flaccid, ribbonlike leaves in the spring and fall, but short broad, stiff ones during the summer (11).

The present work does not fully explain the occurrence of the submersed aerial leaves. Their predominance on plants from northern latitudes suggests that spectral composition, or daylength, may modify the effects of the reduced light intensity. The problems of reduced light intensity and other environmental factors influencing plant growth in extreme northern latitudes have been dealt with elsewhere (32, 36). For aquatics it is probable that the low temperature of the water is a factor. Water uptake may be inefficient owing to a restricted root growth and reduced respiration (23), so that cold water may be physiologically like salt water with higher osmotic values. This suggests that if there is any reduction in availability of water, leaves will be produced on *Hippuris* that tend to be shorter, broader, and of the aerial type with respect to venation, epidermal cell form, etc., as has been found on other plants (4, 8, 19, 21).

The authors are aware of the usefulness of being able to designate the various morphological types of *Hippuris* encountered. However, the terms species, variety, ecotype, and form all denote a genotype, or the possession of an inherited pattern. This may be modified by the environment within certain limits. When individuals belonging to these different genotypes (form, species, ecotype, etc.) are grown in a uniform environment, however, these inherited differences should persist (24, 35). Morphological differences which do not persist when plants are grown under a uniform environment can be considered merely as phenotypic responses to the ecological factors of the environment. The genus *Hippuris* in North America has been subdivided chiefly on the basis of leaf number and leaf shape from the time of Hooker onwards

(2, 17, 18, 25, 28). The number of leaves per whorl has been discussed elsewhere (29), and apices from plants named *H. tetraphylla* L. f. from the MacKenzie River delta, N.W.T., and *H. vulgaris* forma *litoralis* Lindb. f. from Frobisher Bay and Coral Harbour, N.W.T., were included in the samples studied. This suggests that leaf production is the same. The chromosome number is apparently the same in *H. tetraphylla* and *H. vulgaris* (26). *H. tetraphylla* was grown in fresh water in our greenhouse for at least 10 months. It was grown in jars beside others containing *H. vulgaris*. During the first few months there was considerable difference in form. In time this diminished. After they had been growing all winter, the difference was not obvious in the new growth. An assistant, when asked to repot all the material, unwittingly lumped *H. vulgaris* and *H. tetraphylla* together.

Attempts to grow *H. vulgaris* in salt solution were not very satisfactory. Many plastochrons occur before a leaf is mature and the environment stamps its peculiar pattern upon it. However, three or four whorls of leaves whose appearance corresponds to the description of leaves of *H. tetraphylla* have been grown. It should be noted that the shape of leaves No. 3 and 4, Fig. 4, corresponds to this shape. It is doubtful whether the extreme form illustrated in Fig. 12 could be produced in the laboratory.

The aerial material produced by our cultural methods resembles that in Fig. 11. Coarse, long-leafed submersed forms have not been grown in the laboratory, the submersed forms in the laboratory being like the submersed material illustrated in Fig. 14, or that from the far north (Fig. 13). It is not anticipated that extremely coarse material (Fig. 6) could be produced without modification of our cultural methods. Lepage describes *Hippuris tetraphylla* forma *lacunarum* Dutilly & Lepage as having flaccid linear-lanceolate leaves up to 2.5 cm long and 3 mm wide in whorls of three to six (25). This plant was found growing in saline water at a river mouth. Examination of the holotype of this form (Fig. 8) shows that the leaves are similar to the long, flaccid aquatic ones of *H. vulgaris* except for the presence of lateral veins. A collection labelled *Hippuris maritima*, Hell. made at Middle Bay, Labrador, in 1882 (Fig. 9) has similar leaves towards the base and broad aerial leaves towards the apex.

Hippuris montana Ledeb. is listed by Macoun as occurring in turfy places (28). It is typical of wet alpine meadows of northwestern North America. Examination of herbarium sheets, such as that in Fig. 10, shows that the material growing along the stream in Manitoulin Island (see page 1105) and some material cultured in our laboratory fall clearly into the dimensions suggested for this species. According to Abrams, this species has been regarded by some workers as merely a form of *H. vulgaris* L. (2).

Polunin does not follow the tendency to place the morphological variants of *Hippuris* in separate taxonomic categories. He considers that the genus is very plastic and includes the various forms which occur in the Arctic in one polymorphic species, *H. vulgaris* (31). The plasticity of the leaf morphology of this plant demonstrated in this study supports his view. It appears, therefore, that the variations which have been described are best considered as environmental responses of a single species, *H. vulgaris*. The variations described can be thought of as phenotypes of this species.

Acknowledgments

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References

1. ABBE, E. C., RANDOLPH, L. F., and EINSET, J. 1941. The developmental relationship between the shoot apex and the growth pattern of leaf blade in diploid maize. Am. J. Botany, **28**, 778–784.
2. ABRAMS, L. 1951. Illustrated flora of the Pacific States. Stanford University Press, Stanford.
3. ALLSOPP, A. 1954. Experimental and analytical studies of Pteridophytes. XXIV. Investigations on *Marsilea*. 4. Anatomical effects of changes in sugar concentration. Ann. Botany, London, N.S., **18**, 449–461.
4. ALLSOPP, A. 1955. Experimental and analytical studies of Pteridophytes. XXVII. Investigations on *Marsilea*. 5. Cultural conditions and morphogenesis, with special reference to the origin of land and water forms. Ann. Botany, London, N.S., **19**, 247–264.
5. ARBER, A. 1920. Water plants. Cambridge University Press, London.
6. ASHBY, E. 1948. Studies on the morphogenesis of leaves. I. An essay on leaf shape. New Phytologist, **47**, 153–176.
7. CHATIN, A. 1885. Note sur la présence de matière verte dans l'épiderme des feuilles de l'*Hippuris vulgaris*, du *Peplis portula*, des *Jussiaea longifolia* et *J. lutea*, de l'*Isnardia palustris* et du *Trapa natans*. Bull. soc. botan. France, T. **2**, 674–676.
8. COMBES, M. R. 1936. Production experimentale chez une plante immergée des caractères de structure propres aux organes aériens. Comp. rend. **203**, 680–682.
9. COSTANTINE, J. 1885. Observations critiques sur l'épiderme des feuilles des végétaux aquatiques. Bull. soc. botan. France, T. **32**, 83–88.
10. COSTANTINE, J. 1885. Influence du milieu aquatique sur les stomates. Bull. soc. botan. France, T. **32**, 259–264.
11. COSTANTINE, J. 1886. Études sur les feuilles des plantes aquatiques. Ann. sci. nat. Botan. T. **3**, 94–162.
12. CROSS, G. L. 1937. The morphology of the bud and development of the leaves of *Viburnum rufidulum*. Am. J. Botany, **24**, 266–276.
13. DALE, H. M. 1957. Developmental studies of *Elodea canadensis* Michx. 2. Experimental studies on morphological effects of darkness. Can. J. Botany, **35**, 51–64.
14. DUTILLY, A., LEPAGE, E., and DUMAN, M. 1954. Contribution à la flore du versant occidental de la Baie James, Ontario. Contrib. Arctic Inst. Catholic Univ. Am. Washington, D. C., SF.
15. ESAU, K. 1953. Plant anatomy. John Wiley and Sons, New York.
16. GOEBEL, K. 1900. Organography of plants. Vol. I. I. B. Balfour, *Trans*. Oxford Press, London.
17. HOOKER, W. J. 1840. Flora Boreali-Americana, Vol. I. Henry G. Bohn, London.

18. HÜLTEN, E. 1946. Flora of Alaska and Yukon. Hakan Ohlssons Boktryckeri, Lund.
19. JONES, H. 1952. Variation in leaf form in *Callitriche intermedia*. Nature, **170**, 848.
20. JONES, H. 1955. Heterophylly in some species of *Callitriche* with especial reference to *Callitriche intermedia*. Ann. Botany, London, N.S., **19**, 225–246.
21. JONES, H. 1955. Further studies on heterophylly in *Callitriche intermedia;* leaf development and experimental induction of ovate leaves. Ann. Botany, London, N.S., **19**, 369–389.
22. JONES, H. 1956. Morphological aspects of leaf expansion, especially in relation to changes in leaf form. The growth of leaves. F. L. Milthorpe, *Editor*. Butterworths, London. pp. 93–104.
23. KRAMER, P. J. 1949. Plant and soil water relationships. McGraw-Hill Book Co., Toronto.
24. LAWRENCE, G. H. M. 1955. Taxonomy of vascular plants. The Macmillan Co., New York.
25. LEPAGE, E. 1954. Études sur quelque plants américaines. III. Naturaliste can. **81**, 111.
26. LÖVE, A. and LÖVE, D. 1956. Cytotaxonomical conspectus of islandic flora. Acta Horti. Gotoburgensis, **20**, 65–290.
27. MCCALLUM, W. B. 1902. On the nature of the stimulus causing the change of form and structure in *Proserpinica palustris*. Botan. Gaz. **34**, 93–108.
28. MACOUN, J. 1883. Catalogue of Canadian plants. Geological Survey of Canada.
29. MCCULLY, M. E. and DALE, H. M. 1961. Variations in leaf number in *Hippuris*. A study of whorled phyllotaxis. Can. J. Botany, **39**, 611–625.
30. MONTGOMERY, F. H. and RIDDELL, R. T. 1959. An inexpensive plant growth chamber. Can. J. Plant Sci. **39**, 80–81.
31. POLUNIN, N. 1959. Circumpolar arctic flora. Oxford University Press, London.
32. PORSILD, A. E. 1957. Illustrated flora of the Canadian Arctic archipelago. Natl. Museum Can. Bull. 146.
33. SEIDELIN, A. 1910. *Hippuridaceae*, *Haloragidaceae*, and *Callitrichaceae*. The structure and biology of Arctic flowering plants. Copenhagen. pp. 295–332.
34. SVERDRUP, H. U., JOHNSON, M. W., and FLEMING, R. H. 1942. The oceans. Prentice-Hall, Inc., Englewood Cliffs, N. J.
35. TURESSON, G. 1922. The genotypical response of the plant species to the habitat. Hereditas, **3**, 211–350.
36. WARREN-WILSON, J. 1957. Observations on the temperatures of Arctic plants and their environments. J. Ecol. **45**, 499–531.
37. WATSON, R. W. 1942. The mechanism of elongation in palisade cells. New Phytologist, **41**, 206–221.
38. WEIR, C. E. and DALE, H. M. 1960. A developmental study of wild rice, *Zizania aquatica* L. Can. J. Botany, **38**, 719–739.

THE GENUS THERRYA[1]

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Abstract

The genus *Therrya* Sacc. & Penz. is shown to include two distinct species, *Therrya pini* (Alb. & Schw.) von Höhn. and *Therrya fuckelii* (Rehm) Kujala, which have been considered synonymous. The name *Coccomyces strobi* is proposed for the organism occurring on *Pinus strobus* in North America which has been considered synonymous with *Therrya pini* or referred to under invalid or unpublished binomials.

Introduction

Examination of white pine samples collected in Ontario over a period of years often revealed the presence of a discomycetous fungus associated with cankers on leaders of regeneration or on small branches of larger trees (Fig. 12). This organism under the name *Coccomyces pini* Karst. has been reported by various authors (Hedgcock 1932, Hilborn and Markin 1938, and Graves 1913) as associated with, and suspected of causing, twig blight and cankers on eastern white pine.

Comparison of this fungus with specimens on deposit at various herbaria revealed that a nomenclatural problem existed. Many specimens were deposited under a synonym of an unrelated European species, while others were found under various combinations of invalid or unpublished binomials.

In order to resolve this problem this organism was studied in relation to what was then thought to be its European limited counterparts. During the course of this investigation, however, one of the two European entities was collected in Ontario and also found deposited in several Canadian herbaria under wrong or invalid names.

Historical

In 1805, Albertini and Schweinitz described *Xyloma pini*, a discomycete found growing on branches of *Pinus sylvestris*. While their specimen is apparently no longer in existence, their detailed habit sketch of the fungus strongly suggests that this is the organism mentioned by later authors. In 1817, Nees described the same fungus using the name *Hysterium valvatum*, while Schmidt, in Kunze and Schmidt (1817), applied the name *Phacidium pini* to this organism.

Xyloma pini was transferred to *Phacidium pini* (Alb. & Schw.) by Fries (1823) and he noted it had been found on *P. sylvestris* and *Juniperus communis* in Sweden. Later, Link (1833) named this fungus *Pyrenochium pini*.

In 1871, Karsten used the name *Coccomyces pini* (Alb. & Schw.) for this organism, while Saccardo and Penzig (1882) described this fungus as the type

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specimen of a new pyrenomycete genus, applying the name *Therrya gallica*.

Rehm (1888) erected the genus *Coccophacidium* using this organism as the type species, the name thus becoming *Coccophacidium pini* (Alb. & Schw) Rehm.

In 1865, the Tulasnes described a fungus found on *P. sylvestris* under the name *Phacidium pini*, which they mistakenly considered to be synonymous with *Xyloma pini* Alb. & Schw. *et auct.*, while Fuckel (1869) gave a description of the same organism under the name *Phacidium pini* Tul., and cited *Phacidium pini* Fr as a synonym.

This second fungus was described by Rehm (1888) as a variety of *Coccophacidium pini* to which he applied the name *Coccophacidium pini* var. *fuckelii*. He made no mention, however, of the Tulasnes' or Fuckel's descriptions.

In Fungi saxonici, No. 735, Krieger raised the variety *C. pini* v. *fuckelii* to species rank.

In 1912, von Höhnel discussing *Therrya gallica* Sacc. and Penz. stated that this organism belonged to the Pseudophacidiaceae, and was identical with *Coccophacidium pini* v. *fuckelii*. Since the genus *Therrya* predated *Coccophacidium*, the valid name for this fungus was then *Therrya pini* (Alb. & Schw.) von Höhnel.

Ellis and Everhart (1901) employed the name *Phacidium crustaceum* (Curtis) Ell. & Ev. for the organism found on *Pinus strobus* in North America. Samples of this fungus were issued as specimen 1554 in the Fungi columbiani but this combination was based on an unpublished binomial, *Coccomyces crustaceum*, in the Curtis herbarium. It was also distributed as No. 455 in North American Fungi under the name *Phacidium crustaceum* Curtis. The note on this packet indicates this is also an unpublished binomial in the Curtis herbarium.

About 1902, E. J. Durand started to use the name *Coccophacidium crustaceum* (Curtis) Durand for specimens of the North American organism on *Pinus strobus* deposited in his own herbarium. It is difficult to be sure of the date, but Durand's specimen No. 1794 is the first one to have this name on the packet, not as a correction, but as the original name. Two earlier collections by Durand, one in 1895 and one in 1902, originally labelled *Phacidium crustaceum* (Curtis) E. & E. and *Phacidium crustaceum* (Curtis) have the name *Phacidium* crossed out, and *Coccophacidium* inserted in Durand's own handwriting. There are subsequent collections in Durand's herbarium under the name *Phacidium crustaceum* Berk. & Curt. or Curtis, but these all appear to have been collected and determined by other individuals. All of Durand's subsequent collections or determinations are as *Coccophacidium crustaceum* (Curtis) Durand.

Although this name was apparently never validly published in any of Durand's available papers but is an unpublished combination based on an unpublished species in the Durand herbarium,[4] it came into general use for North American material, and was listed by Seymour (1929) on page 39.

In 1917, von Höhnel stated that the North American material cited as *Phacidium crustaceum* (Curt.) Ell. & Ev. (= *Coccomyces crustaceus* Curtis) had not been described. Furthermore, he noted that specimen No. 1554 in Fungi

[4]This information kindly supplied by Dr. E. K. Cash and verified by Dr. R. P. Korf.

columbiani was nothing more than an undeveloped form of *Therrya pini* (A. & S.) von Höhn. (= *Coccophacidium pini* (A. & S.) Rehm).

At this point then, the valid name for all the European and North American material appeared to have been *Therrya pini* (Alb. & Schw.) von Höhn. In 1950, Kujala transferred *Coccophacidium pini* v. *fuckelii* to *Therrya fuckelii* (Rehm) Kujala, thus accepting Rehm and Kreiger's views that this organism was distinct from *Therrya pini* (Alb. & Schw.) von Höhn. Unfortunately, however, Kujala was apparently unaware of von Höhnel's transfer of the two organisms to *Therrya pini*, and he thus apparently also transferred *Coccomyces pini* (Alb. & Schw.) Karst. (*Coccophacidium pini* (Alb. & Schw.) Rehm) to *Therrya pini* (Alb. & Schw.) Kujala. He also made the mistake of assuming the North American organism on *Pinus strobus* was identical with *Therrya pini*.

Gremmen (1960*a*) while tentatively accepting Kujala's transfers stated that: "Rehm (1896) described this fungus as *Coccophacidium pini* v. *fuckelii* separating it from *C. pini*, but it is very doubtful whether there is a real difference between them". In a more recent paper Gremmen (1960*b*) agrees with the separation of *Therrya pini* from *Therrya fuckelii*, but still includes the North American material with *Therrya pini*.

Discussion

Examination of numerous examples of the European and North American organisms have led the writers to the conclusion that there are three separate entities involved in this complex. These are *Therrya pini* (Alb. & Schw.) von Höhn; *Therrya fuckelii* (Rehm) Kujala, and the previously undescribed North American organism, for which the name *Coccomyces strobi* is proposed.

Von Höhnel's misinterpretation of the relationship of these organisms appears to be the cause of the confusion which has existed, and it would seem wise to discuss our interpretation with reference to his views.

As mentioned previously, von Höhnel (1912) stated that *T. gallica* was not a pyrenomycete as described by Saccardo and Penzig (1882), but a discomycete and synonymous with *Coccophacidium pini* v. *fuckelii*. Furthermore he believed that *C. pini* was simply an immature form of *C. pini* v. *fuckelii*. He stated that: "Kreiger hat diese Varietät zur Art erhoben, allein Rehm hat gefunden, das auf deselben Exampleren die kleineren Apothecien sporen wie *C. pini* zeigten und die grösseren (offenbar besser entwickelten) Sporen hatten wie die Varietät *Fuckelii*. Trotzdem schliesst sich Rehm Kriegers ansich an. Offenbar stellt indes die var. *Fuckelii* nur die gut entwickelte Form dar und kann daher nich einmal als Varietät betrachtet werden." This implies that Krieger and Rehm had made their respective nomenclatural changes on the basis of one sample, although the note on the packet of Fungi saxonici 735 (FH) clearly indicates that Krieger made his decision on the basis of an examination of about 150 samples which he apparently found to be quite constant. Thus, following von Höhnel's reasoning, the valid name for the European material had to be *T. pini* (Alb. & Schw.) von Höhn., the mature form of which, on the basis of the European material available to the writers, is rarely encountered.

This interpretation is, on the basis of this study, quite in error.

Examination of both mature and immature fruiting bodies of *T. fuckelii* collected in Ontario has never revealed the presence of spores resembling those of *T. pini*. The spores are, at maturity, typically straight to acerose, acute at both ends, 4–12 celled, with the end cells being elongated to form an apiculus (Figs. 1, 13). This apiculus is present even in the small, one-celled, immature spores (Fig. 1), clearly indicating that the apiculus is a structure which develops early in the ontogeny of the spore, and thus we cannot account for the large, many-celled spores of *T. pini* lacking an apiculus, if von Höhnel's view were correct. The spores of *T. pini* in all the European material examined by the authors are needle-shaped and wider at one end. They taper sharply at the wide end but much more gradually at the other end, and are curved to sinuous or only slightly bent (Figs. 2, 14). In no case was an apiculus found on any of the spores of any of the material of *T. pini* examined by the authors. Furthermore, fruiting bodies of both organisms in all stages of development (from immature to nonerumpent closed forms, to erumpent fully opened forms) were examined, thus ruling out the possibility of having examined only immature stages.

In addition, *T. pini* has 8 spores lying coiled in each ascus (Fig. 6), while *T. fuckelii* has only 4 spores per ascus, and they lie parallel to the long axis (Fig. 5).

The paraphyses of both fungi are swollen at the tips, and encrusted with a dark, brown material which appears to impregnate the walls (Figs. 4, 13, 14). Occasionally, however, the encrustation is lacking on the paraphyses of *T. fuckelii*, resulting in an appearance shown on the paraphysis in Fig. 13, and all paraphyses in Fig. 3.

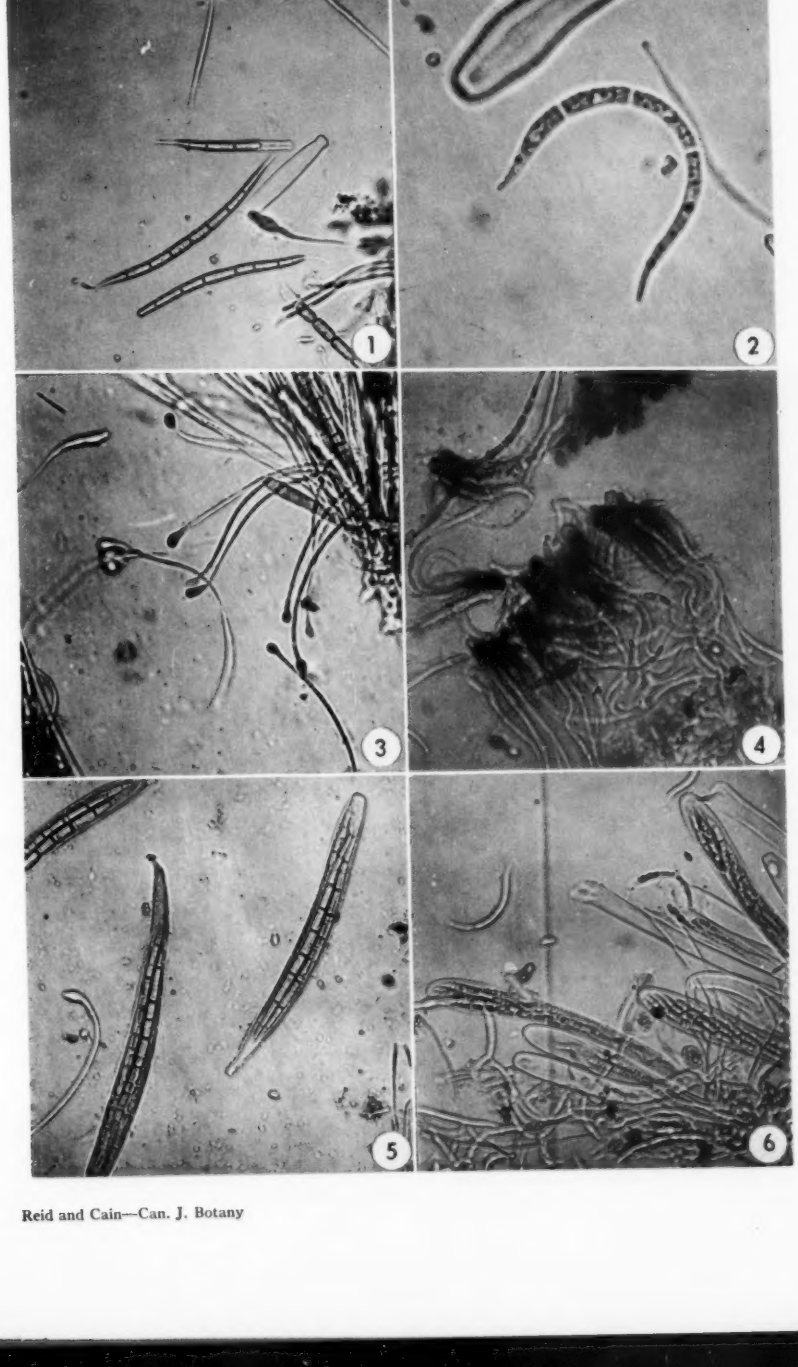
In section, the fruiting bodies are quite different. *T. pini* has a relatively thinner, persistent hypothecium, which is subtended by a rather well developed subiculum composed of dark thick-walled mycelium (Fig. 9). On the other hand, *T. fuckelii* has a much thicker, better-developed hypothecium, which often breaks down at maturity leaving a gap below the hymenial layer. The subiculum is scarcely, or not at all developed, the hypothecium resting directly on the underlying bark (Fig. 11).

Coccomyces strobi is quite different from either of the above-mentioned organisms. The spores at maturity are never more than one-celled, continuous, tapering below, blunt above, nearly straight, but often with a slight hook above (Figs. 7, 15). Even the largest spores are rarely one-half as long or one-half as wide as the spores of the other two fungi. Furthermore, the spores of *C. strobi* are enclosed in a mucilaginous sheath, while those of the other organisms are not.

Von Höhnel (1917), in support of his theory that *C. strobi* was merely an immature form of *T. pini*, wrongly stated that the spores of the North American material were still enclosed in the asci, and therefore only seemed thinner. He

FIG. 1. Ascospores of *T. fuckelii*, ×330.
FIG. 2. Ascospores of *T. pini*, ×750.
FIG. 3. Nonencrusted paraphyses of *T. fuckelii*, ×330.
FIG. 4. Typical encrusted paraphyses of *T. pini* and *T. fuckelii*, ×330.
FIG. 5. Asci and ascospores of *T. fuckelii*, ×350.
FIG. 6. Asci and ascospores of *T. pini*, ×350.

PLATE I



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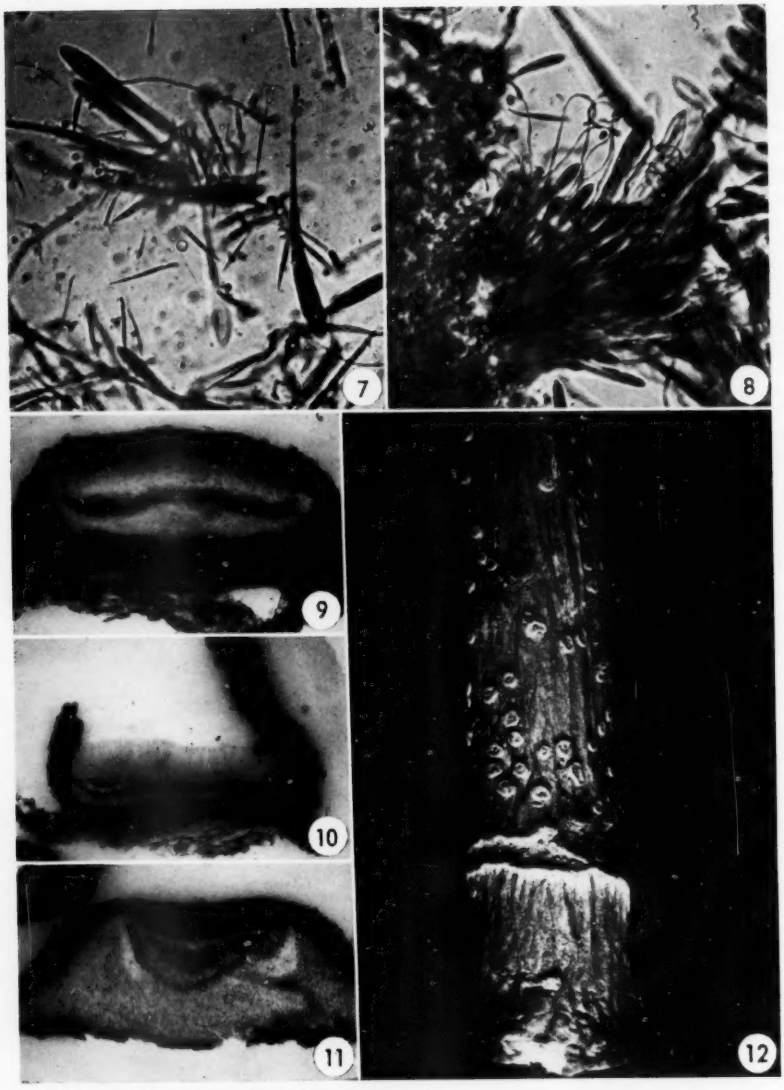

FIG. 7. Asci and ascospores of *C. strobi*, ×330.
FIG. 8. Asci and paraphyses of *C. strobi*, ×330.
FIG. 9. Section of *T. pini*, ×45.
FIG. 10. Section of *C. strobi*, ×45.
FIG. 11. Section of *T. fuckelii*, ×45.
FIG. 12. Cankered terminal twig of *Pinus strobus* bearing mature fruiting of *C. strobi*, ×3.

also noted that specimen No. 1924 in Krypt. exs. Museum Vindob. was a good example of what a mature specimen on white pine looked like. In conclusion, referring to specimen 1924, he stated: "Er sieht hier dem amerikanischen Exemplar ganz gleich und ist nur etwas grosser". It appears that this statement must be the one which has resulted in much of the North American material being labelled *Coccophacidium pini* (Alb. & Schw.) Rehm, although why von Höhnel's transfer of this organism to *T. pini* was not noted is surprising.

In reality, specimen 1924 is a good example of *T. pini* on the European *P. strobus*. In gross appearance it resembles *C. strobi* on the North American white pine, but microscopically and in section it clearly resembles *T. pini* on *P. sylvestris*.

The paraphyses of *C. strobi*, in contrast to those of *T. pini* and *T. fuckelii*, are slender, filamentous, coiled, or recurved, but neither swollen nor encrusted at the tips or fused together at the tips to form an epithecium (Figs. 8, 15).

FIG. 13. Camera lucida drawing of asci, ascospores, and paraphyses of *T. fuckelii*.

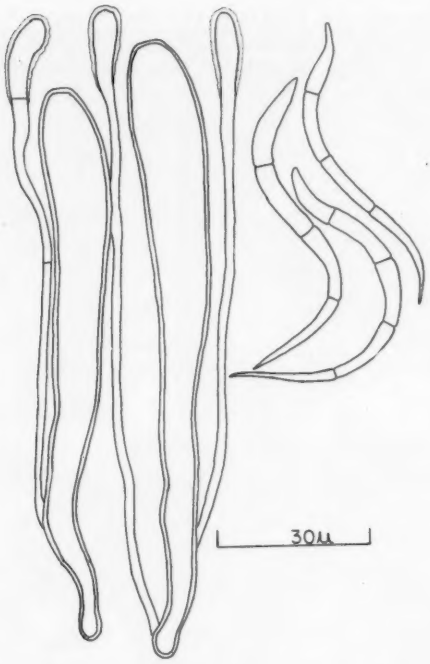

FIG. 14. Camera lucida drawing of asci, ascospores, and paraphyses of *T. pini*.

The asci of *C. strobi* are rounded to pointed above (Figs. 7, 15), while those of *T. pini* and *T. fuckelii* are blunt above (Figs. 5, 6, 13, 14).

The fruiting bodies of *C. strobi* have a well-developed hypothecium, similar to that found in *T. pini*, which is subtended by a very well developed, black subiculum (Fig. 10).

In view of these findings, the authors believe that there is ample evidence to support their view that the three species have actually been confused in this complex. In fact, in view of the fundamental differences noted between *T. pini* and *T. fuckelii*, there is some doubt whether these fungi should properly be placed in the same genus. However, in view of their past history, and the lack of detailed developmental studies, it has been decided to recognize them as being congeneric for the present.

At this point consideration should be given to two other organisms, *Coccomyces cembrae* Rehm and *Coccomyces juniperinum* Karst.

The name *C. cembrae* Rehm has been used for some specimens of the fungus found on white pine in North America. Examination of these collections shows that they are identical with *C. strobi*, and an examination of the type of *C. cembrae* showed it was distinct from *C. strobi*. Wehmeyer (1950) identified an organism found on *Larix laricina* in Colchester County, Nova Scotia, as

C. cembrae. Comparative examination of this specimen (Wehmeyer herbarium 2,082), with other material, indicates it is a distinct, undescribed species, and only the paucity of material prevents the authors from describing it as new.

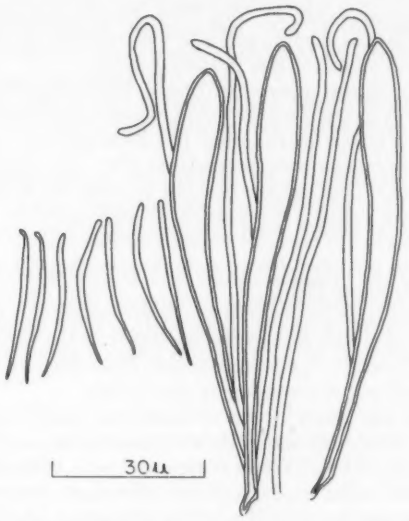

FIG. 15. Camera lucida drawing of asci, ascospores, and paraphyses of *C. strobi.*

Coccomyces juniperinum Karst. (= *Colpoma juniperinum* Cooke & Pk.) has been reported on *Juniperus virginiana* and *Abies balsamea* in New York State, but this organism, although apparently closely related to *C. strobi*, is not identical with it.

The disposition of the genus *Therrya* is of some interest. In 1932, Nannfeldt, apparently not having been aware that the specific epithet *gallica* was invalid and should have been *pini*, stated that the genus *Therrya* along with its type species *T. gallica* did not belong in the Phacidiaceae. In 1926, von Höhnel stated that "Die beiden Gattungen *Therrya* Sacc. und *Colpoma* Wallr. stehen einander sehr nahe und sind eigentlich nur dadurch von einander verschieden, dass die Fruchtkörper von ersterer mehr rundlich sind, lappig aufspringen und die Decke schliesslich ganz abwerfen, während letztere längliche Fruchtkorper aufweist, die mit einem Längsspalt aufreissen und die Decke nicht abwerfen." He goes on to say that the two genera are similar in that they have the same imperfect stage. Von Höhnel noted that Tulasne (1865) had described the imperfect stage of *T. pini* (Alb. & Schw.) von Höhn., and that: "Diese Nebenfrucht ist aber nicht häufig zu finden. Ich traff sie nur in dem Ellis et Everhart, Fungi Col. Nr. 1554 unter den Namen *Phacidium crustaceum* (Curt.) E. & Ev. auf *Pinus strobus* ausgegebenen Exemplare". Thus the imperfect stage von Höhnel described as *Colpomella pini* von Hohn. would actually be related to *Coccomyces strobi*, and thus have no connection with the genus *Therrya*.

He was apparently unaware that Karsten (1890) had given the imperfect stage of *T. pini* as *Fusicoccum pityophilum* (Sacc.) Karst. Von Höhnel, therefore, was actually confirming the close relationship of *C. strobi* to the genus *Colpoma*. In Gremmen's most recent paper, however, he reports having obtained *Colpomella pini* from ascospores of *T. pini*. Thus, the relationship of this imperfect to the above ascomycetes is confused, and will only be clarified when *C. strobi* is cultured, a feat which so far has proved difficult.

In 1942, because of the above-mentioned error, Terrier stated that von Höhnel had placed the genus *Therrya* near *Colpoma*. Reference was also made to Nannfeldt's transfer of the genus from the Phacidiaceae, and he goes on to note that: "*Coccophacidium pini* possède en effet des spores totalement différentes de celles, que l'on rencontre dans le reste de la famille. Elles sont pluriseptées et chaque cellule est munie de grandes guttules, en autre les deux extrémités sont effilées. Les paraphyses sont de même d'un type inconnu chez les Phacidiacées, elles sont renflées au sommet qui apparaît subsphérique, mais non septé comme l'indique Rehm."

From this it appears quite clear that Terrier was in doubt as to the relationship of this genus to the rest of the members of the family Hypodermataceae sensu Terrier, to which it would probably have been assigned. No mention of this genus is made in his summary of the family.

Terrier proposed the erection of two families, the Hypodermataceae and the Rhytismaceae, within the order Hypodermatales sensu Terrier. This order contained only ascohymenial ascomycetes and was thus distinguished from his order Phacidiales which contained only locular ascomycetes comprising the family Phacidiaceae sensu Terrier. On the other hand, Müller and von Arx (1954) divide the Phacidiales into three families, the Hypodermataceae, the Phacidiaceae, and the Cryptomycetaceae. According to the descriptions of their families, the genus *Therrya* cannot be properly placed in any of the families described.

The authors feel the disposition of this genus is a difficult problem which could only be solved by a detailed comparative study of the genus *Therrya* with its related forms. As this is beyond the scope of the present study, it is felt that it is best not to assign the genus to any one family for the present.

Description of Species

THERRYA PINI (Alb. & Schw.) von Höhn., Fragmente zur Mycologie, **14**, No. 778 (1912); Figs. 2, 4, 6, 9, and 14

Xyloma pini Alb. & Schw., Consp. fung. Nisk., p. 60 (1805).

Hysterium valvatum Nees, Das System der Pilze und Schwämme, p. 282 (1817).

Phacidium pini Schmidt., Mykologische Hefte, **I**, 30 (1817).

Phacidium pini (Alb. & Schw.) Fr. Syst. Myc. **II**, 573 (1823).

Pyrenochium pini Lk., Handb. z. Erk. d. Gew. **III**, 385 (1833).

Coccomyces pini (Alb. & Schw.) Karst., Mycologia fennica pars prima, **19**, 254 (1871).

Therrya gallica Sacc. & Penz., Michelia, **II**, 604 (1882).

Coccophacidium pini Rehm, Rabenhorst's Krypt.-flora, **I** (3), 98 (1888).

Therrya pini (Rehm.) Kujala, Comm. Inst. Forestry Inst. **38**, 49 (1950).

Apothecia 1.5 to 3 mm wide, scattered or clustered, oval, closed, immersed, then erumpent rupturing the periderm irregularly. Opening by means of longitudinal fissures or irregular lobes, to expose the dark brown to black epithecium. Excipulum dark brown to black, membranaceous to carbonaceous, strongly developed, and remaining as lobes surrounding the apothecium. Composed of angular to irregularly shaped thick-walled cells. Hymenium colorless to light yellow-brown. Hypothecium colorless, composed of irregularly shaped cells, and often containing crystalline material.

Subiculum dark, composed of thick-walled brown hyphae and irregularly shaped cells, and incorporating some of the underlying bark tissue in its structure.

Asci clavate, blunt above, 90–135×12–16.5 μ, 8-spored. Paraphyses, filamentous, swollen to 6 μ, encrusted at the tips with a dark substance intermixed with asci and projecting beyond them, adhering together to form an epithecium.

Spores hyaline, 50–80×2.5–4.5 μ, needle-shaped, much wider at one end, tapering sharply at the wide end, much more gradually at the lower end, curved, sinuous, sigmoid to only slightly bent, at first single-celled, later becoming 4- to 8-celled, lying coiled spirally in the ascus, or very rarely parallel in the long axis of the ascus.

Exsiccati.—Jacz., Kom. et Transch., Fung. ross. 141 (as *Coccophacidium pini*), DAOM; Thüm., Myc. univ. 179 (as *Phacidium pini*), DAOM, FH; Fck., Fungi rhen. 1095 (as *Coccophacidium pini*) TRTC, UPS; Krieg., Fung. sax. 734 (as *Coccophacidium pini*), S, FH, TRTC; Krypt. Exs. Mus. Palat. Vindob. 1924 (as *Coccophacidium pini*), DAOM, FH. Kze., Fung. sel. 561 (as *Phacidium pini*), S, FH; Rab., Fung. eur. 3568 (as *Phacidium pini*), FH, UPS; Rehm, Asc. 625 (as *Coccomyces pini*), S, FH; Roum., Fung. gall. 1268 (as *Phacidium pini*), DAOM.

Specimens examined.—Europe.

On *Pinus sylvestris.* Sweden: (*Therrya pini*) Uppland, March 10, 1884, L. Romnell (Flora suecica UPS); Uppland, July 3, 1942, E. Åberg (Fungi suecici UPS). (*Therrya fuckelii*) Gästrikland, Fungi suecici 12826 (UPS). (*Phacidium pini*) Smaland, ex Fries Herb. (UPS); Uppland, ex Fries Herb. (UPS). Germany: (*Phacidium pini*) leg Kunze, ex Fries Herb. (UPS); Leipzig, Saxoniae, ex Rehm Herb. (S). (*Therrya gallica*) Wien, July 3, 1916 ex Herb. v. Höhn. (FH). (*Coccomyces pini*) Falkenstein, Oberpfalz, Aug. 1892, ex Rehm Herb. (S). France: (*Therrya gallica*) J. J. Therry (type?) ex herb. v. Höhn. 4545 (FH). (*Phacidium pini*) Caen, leg. Le Normand.

On *Pinus strobus.* France: (*Phacidium pini strobi*) leg. Chaillet, ex Fries Herb. (UPS). (*Xyloma pini strobi*) ex Fries Herb. (UPS).

THERRYA FUCKELII (Rehm) Kujala, Comm. Inst. Forestry Inst. **38**, 4 (1950); Figs. 1, 3, 5, 11, and 13

Phacidium pini Tul., Selecta Fungorum Carpologia, **III**, 135 (1865); Symbolae Mycologicae, 263 (1869).

Coccophacidium pini var. *fuckelii* Rehm, Rabenhorst's Krypt.-flora, **I** (3), 99 (1888).

Coccophacidium fuckelii (Rehm) Krieger, Fungi saxonici No. 735 (1891).

Apothecia, scattered or clustered, 1–3 mm in diameter, immersed, black, erumpent at maturity, rupturing the periderm irregularly. Opening irregularly by lobes or splitting longitudinally to expose the dark brown to black epithecium. Excipulum dark brown to black, membranaceous to carbonaceous, strongly developed with fragments of the outer bark remaining adhered to the outer portion. Cells of excipulum angular to irregularly shaped, dark, thick-walled. Subiculum lacking, the hypothecium resting directly on the underlying bark. Hypothecium thick, colorless to white to yellow brown at maturity, firm in early stages of maturation, and composed of irregularly shaped cells, which contain numerous crystals, often disintegrating at maturity to leave a cavity below the hymenium.

Hymenium yellowish to occasionally dark brown in section.

Asci (105)120–157×(10)12–15(17) μ, club-shaped to nearly cylindrical, broadly rounded and blunt above, broadest near middle, tapering gradually below to a short stipe, 4-spored. Paraphyses swollen at the tips to 6 μ, agglutinated to form the dark epithecium, intermixed with asci. Tips of paraphyses appear to be encrusted with a dark substance, which gives the dark color to the epithecium. Occasionally this substance is lacking, and the tips appear hyaline.

Ascospores (64.5)75–100(110)×3.0–4.5(5.5) μ, straight to slightly curved, acerose acute at both ends, with an apiculus 12–18 μ long at each end, and present on even very young cells, hyaline, 1-celled when young, becoming 8–12 celled with the formation of the septa, slightly constricted at the septum, and lying parallel in the ascus. Apiculus broad at base, abruptly contracted to long spine.

Exsiccati.—Krieg., Fung. sax. 735 (as *Coccophacidium fuckelii*), TRTC, FH; Fungi sax. 735B (as *Coccophacidium fuckelii*), FH.

Specimens examined.—Canada. Ontario: (*Therrya fuckelii*) Tottenham, Simcoe Co., TRTC 36,470; Tottenham, Simcoe Co., MFB6049. (*Coccophacidium pini*) Bear Island, Timagami, TRTC 9,145; Bear Island, Timagami TRTC, 9,137. (*Coccophacidium pini* v. *fuckelii*) Bear Island, Timagami, TRTC 11,570. All on *Pinus resinosa.*

United States. Michigan: (*Coccophacidium pini* v. *fuckelii*) Cross Village, DAOM 20,762. On *Pinus resinosa.*

Europe. Sweden: (*Therrya fuckelii*) Hälsingland, Flora suecica 14,764 (UPS); Gästrikland, Flora suecica 12,826 (UPS). (*Therrya sp.*) Östergötland, Flora Suecica 15,021 (UPS). (*Therrya gallica*) Uppland, Flora suecica 9177; Gotland, Hemse s:n, Oxarfoe a, Aug. 4, 1897, Flora suecica (UPS). (*Therrya pini*) Södermanland, Södertalje, Näset, Aug. 3, 1949, Flora suecica (UPS). Hungary: (*Phacidium pini*) Scepusii, ex Fries Herb. (UPS). (*Coccomyces pini* corrected to *Phacidium pini*) Magyarhon viranyabol, ex Fries Herb. (UPS). France: (*Xyloma pini*) leg. Chaillet, ex Fries Herb. (UPS). (*Phacidium pini*) Voiges, leg. Quélet (UPS). Germany: (*Coccomyces pini*) im Wald bei Obersaal, Kelthonia, ex Rehm Herb. (S). Finland: (*Therrya pini*) Ta: Tammela s:n, Mustiala, May 3, 1872, Flora fennica (UPS).

Country unverified. (*Hysterium valvatum*) leg. Nees, ex Fries Herb. (UPS). (*Coccophacidium pini*) leg. Kmet (?) ex Rehm Herb. (S). (*Coccomyces pini* v. *fuckelii*) Fuckels u Karstens Pilz, ex Rehm Herb. (S). All on *Pinus sylvestris.*

Coccomyces strobi sp. nov.; Figs. 7, 8, 10, 12, and 15

Apotheciis dispersis vel aggregatis, 1–2 mm diam., initio immersis, dein erumpentibus, papilla parva media praeditis, atro-brunneis vel atris, orbiculatis. Hymenio viridulo-luteo vel luteo-brunno. Hypotheceo subhyalini, ca. 100 μ crasso. Excipulo atro-brunneo vel atro, duro, crustoso, fissuris radiatis, inaequalibus dehiscenti. Subiculo ca. 150 μ crasso, atro, firmo, e hyphis et cellulis atris, angulatis, crasse tunicatis constituto. Ascis clavatis, octosporis, 70–120(125)×6–9(10.5) μ, superne anguste rotundatis. Paraphysibus hyalinis, filiformibus, superne recurvatis, hand agglutinatis. Ascosporis fasciculatis, clavato-filiformibus non-septatis, 27–40.5(48)×1–2 μ, leviter curvatis, superne latioribus, strato mucoso hyalino obductis.

Apothecia scattered or clustered, 1–2 mm in diameter, immersed when young, becoming erumpent, with a small central papilla, rupturing the periderm irregularly, dark brown to black at maturity, circular, opening irregularly by longitudinal or irregular clefts to expose the asci. The outer cells of the bark remain adherent to the outer layer of the excipulum.

Hymenium greenish-yellow, to light yellow-brown. Hypothecium mainly colorless, ca. 100 μ in thickness, composed of angular to irregularly shaped cells, with some browning in layers throughout, containing numerous crystals.

Excipulum dark brown to black, hard, crusty, composed of thick-walled pentagonally to hexagonally shaped cells.

Subiculum thick, ca. 150 μ in thickness, black, firm, composed of thick-walled, dark hyphae and dark, thick-walled, angular cells.

Asci clavate, broadest near apex, conspicuously narrowed above, acutely rounded at apex, gradually tapering below to the well-defined foot, 70–120 (125)×6–9(10.5) μ, 8-spored.

Ascospores 1-celled, filiform-clavate, continuous 27.0–40.5(48)×1–2 μ, broadest near upper end, broadly rounded at apex, tapering gradually below to pointed base, nearly straight, but slightly hooked above, surrounded by a distinct mucilaginous sheath.

Paraphyses colorless, filamentous, curled, coiled, or recurved at their tips, tending to form an epithecium, but not agglutinated at their tips.

Type specimen:—Midhurst, Simcoe County, Ontario, June 4, 1951. On *Pinus strobus.* R. F. Cain TRTC 23,578.

Host:—Pinus strobus.

Exsiccati.—Ell. N. Am. Fungi 455 (as *Phacidium crustaceum*), TRTC; Ell. & Ev. Fungi columbiani 554 and 4743 (as *Phacidium crustaceum*), TRTC; Farlow Herb. Reliquae Farlowianae 108 (as *Coccophacidium pini*), TRTC.

Specimens examined.—Canada. Nova Scotia: (*Coccophacidium pini*) Truro, TRTC, ex Wehmeyer Herb. 1674. (*Coccophacidium crustaceum*) Kentville, DAOM 29511. New Brunswick: (*Coccophacidium crustaceum*) woodlot U.N.B., DAOM 56809; Fredericton, DAOM 19,544. Ontario: (*Coccophacidium crust-*

aceum) Ottawa, DAOM 20,761; P.F.E.S., DAOM 19,759; Ottawa, DAOM 20,261; P.F.E.S., DAOM 19,759; Chalk River, TRTC 23,920; Brant Co., TRTC 22,821; Midhurst, TRTC 23,578; Brant Co., TRTC 22,817; Simcoe Co., TRTC 24,481; Telfer Twp. TRTC 22,823. (*Coccophacidium pini*), Law Twp., MFB 6024; Latchford P.O., MFB 5,637; Appleby Twp., MFB 5,433; Inwood Twp., MFB 5,829; Delamere Twp., MFB 5,417; Barber Twp., MFB 5,860; Nairn Twp., MFB 5,437; Uxbridge Co. Forest, MFB 4,370; Vivian Co. Forest, MFB 4,968; Nairn Twp., MFB 4,716; Egremont Twp., MFB 5,425; Timagami, TRTC 3,553; Nashville, TRTC 24,588, and 24, 442.

United States. New York: (*Coccophacidium pini*) Cold Spring Harbor, ex Herb. of Howard J. Banker 3,876; Ithaca, ex Herb. J. H. Miller 3,001. (*Coccophacidium crustaceum*) Ithaca, EJD 1,347; East Ithaca, EJD 1,352; Churchville, EJD 1,794; West Albany EJD 6,249; Guilderland EJD 6,250; N. Greenbush, EJD 6,251; Portage, EJD 6,252; South Hill, EJD 6,805 all Herb. Elias J. Durand (all CUP); Ithaca, 14,950 and Eagle Hill, 21,879, Atkinson Local Collection, (CUP). (*Phacidium crustaceum*) New York EJD 3,379 and 10,130, Herb. Elias J. Durand (CUP). New Hampshire: (*Coccophacidium crustaceum*) Waterville, FP 50,992 ex BPI. Connecticut: (*Coccophacidium crustaceum*) Portland, FP 58,521 ex BPI. Massachusetts: (*Coccophacidium pini*) Canton, June 2, 1934, D. H. Linder Farlow Herb. (TRTC); Canton, July 10, 1933, D. H. Linder Farlow Herb. (DAOM 24,778). (*Phacidium crustaceum*) Magnolia, April, 1896, ex Herb. W. G. Farlow (DAOM 24,871, Herb. Elias J. Durand, 7,859 CUP); Newton, Jan. 1879, W. G. Farlow (Herb. Elias J. Durand 7861 CUP). (*Coccophacidium cembrae*) Fair Grounds, Nantucket, Massachusetts Fungi Nan. 121 (MASS). New Jersey: (*Phacidium crustaceum*) Newfield, July, 1885, J. B. Ellis (Herb. Elias J. Durand 7,860 CUP). Minnesota: (*Coccophacidium crustaceum*) Taylers' Falls, Oct. 5, 1918, E. J. Durand (Herb. Elias J. Durand 10,675 CUP). Wisconsin: (*Coccophacidium crustaceum*) Madison, May 31, 1951, H. C. Greene (ex Herb. Univ. Wisconsin); Madison, May 2, 1951, H. C. Greene 1523 (ex Herb. Univ. Wisconsin).

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References

De Albertini, J. B. and Von Schweinitz, L. D. 1805. Consp. fung. in Lusatiae. Lipsiae. pp. 1–376.

Fries, E. M. 1823. Systema mycologicum Lund. Vol. II. p. 573.

FUCKEL, L. 1869–70. Symbolae mycologicae Beiträge zur Kenntnis der rheinischen Pilze. 263. *In* Fahrb. d. Nassauischen Ver. f. Natur. Vols. 23 and 24.
GRAVES, A. H. 1913. Notes on diseases of trees in the southern Appalachians. I. Phytopathology, **3**, 129–139.
GREMMEN, J. 1960*a*. A contribution to the mycoflora of pine forests in the Netherlands. Nova Hedwigia, **I**, 252–288.
——— 1960*b*. Conifer inhabiting fungi I. *Therrya pini* Penz. et Sacc., *Phacidium lacerum* Fr. and *Pseudophacidium ledi* (A. et S.) Karst. Nova Hedwigia, **II**, 547–553.
HEDGCOCK, G. G. 1932. Notes on distribution of some fungi associated with diseases of conifers. Plant Disease Reptr. **16**(4), 28–42.
HILBORN, M. T. and MARKIN, F. L. 1938. List of causes of fungus and bacterial plant diseases in Maine to 1936 inclusive. Plant Disease Reptr. Suppl. No. **105**. pp. 1–60.
KARSTEN, P. A. 1871. Mycologia fennica. Pars. prima. discomycetes Bidr. t. Kännedom af Finlands Nat. o. Folk, **19**, 254.
KUJALA, V. 1950. Über die kleinpilze der konniferen in Finnland. Comm. Inst. Forestry Inst. **38**, 48–49.
KUNZE, G. and SCHMIDT, J. R. 1817. Mykologische Hefte. Leipzig. Vol. I. p. 30.
LINK, D. H. 1833. Handbuck zur Erkennung der nutzbarsten und am haufigsten vorkommender Gewachse. Vol. III. p. 385.
NANNFELDT, J. A. 1932. Studies uber die morphologie und systemmatik der nicht-lichenisierten inoperculaten Discomyceten. Nova Acta Regiae Soc. Sci. Upsaliensis, Ser. IV, **8**(2), 1–368.
NEES V. ESSENBECK, C. G. 1817. Das System der Pilze und Schwamme. Wurzburg. p. 282.
REHM, H. 1888. Die Pilze Deutschlands, Oesterreichs und der Schweiz. Rabenhorsts Kryptogrammen-flora. Bd. I, Abt. 3, 97–99.
SACCARDO, P. A. and PENZIG, O. 1882. Michelia. Vol. II. p. 604.
SEYMOUR, A. B. 1929. Host index of the fungi of North America. Harvard University Press.
TULASNE, L. R. and TULASNE, C. 1865. Selecta Fungorum Carpologia. Vol. III. pp. 135–136.
VON HÖHNEL, F. 1912. Fragmente zur Mycologie, **15**. No. 778.
——— 1917. Ann. Mycol. Notitiam Sci. Mycol. Univ. **15**, 326.
——— 1926. Uber die nebenfruchtformen von *Therrya* Sacc. und *Colpoma* Wallroth. Mitt. botan. Inst. Technol. Hochsh. Wien, III (1), 13–16.
WEHMEYER, L. E. 1950. The fungi of New Brunswick, Nova Scotia, and Prince Edward Island. Natl. Research Council Can. No. 1890. pp. 1–150.

STUDIES ON THE CYTOCHROME OXIDASE AND OXIDATION PATHWAY IN UREDOSPORES OF WHEAT STEM RUST[1]

G. A. WHITE[2] AND G. A. LEDINGHAM

Abstract

Electron transport to oxygen in a particulate fraction from uredospores of *Puccinia graminis* var. *tritici* occurs through a series of carriers similar to those of other fungi and higher plants.

Experiments with various enzyme inhibitors and measurements of the oxygen affinity of respiration have shown that cytochrome oxidase mediates the final step in the sequence of electron transfer. The enzyme was localized in a fraction sedimenting at 20,000*g* and was typically inhibited by cyanide, azide, and CO-dark, the latter inhibition being light-reversible. Other enzymes present were succinic-cytochrome *c* reductase, DPNH- and TPNH-cytochrome *c* reductase, dye reductase, malic dehydrogenase, isocitric dehydrogenase, and glycerol-1-phosphate dehydrogenase. Particulates failed to oxidize DPNH unless an electron acceptor was added. An increase in the activity of several of the respiratory enzymes was noted upon spore germination.

Succinic-cytochrome *c* reductase was only partially sensitive to Antimycin A, HOQNO, and the naphthoquinone, SN 5949. These compounds markedly inhibited a labile portion of the DPNH-cytochrome *c* reductase activity but had little effect on the stable activity remaining in aged particles. Menadione, but not vitamin K_1, stimulated electron transfer. Antimycin A and SN 5949 virtually blocked spore respiration suggesting a "Slater-type" factor in the intact pathway of oxidation.

Introduction

The constitution and role of cytochrome-mediated reactions in tissues of higher plants are well established (16, 30). By contrast, relatively few studies have been made on the nature of the electron transport chain in fungi (4, 6, 21, 26, 32). Most of these organisms appear to respire via flavoprotein and a typical sequence of cytochromes as in higher plants. Cytochrome oxidase is implicated in the terminal respiration of a number of fungi (4, 8, 21, 26). Some (26, 35) are known to respire substrate by the citrate oxidation cycle and its associated enzymes of electron transport.

Almost all enzyme studies on fungi which are obligate parasites have been with wheat rust spores. Allen (1) detected cytochrome oxidase and malic dehydrogenase but no ascorbic acid oxidase in the rust spore. The data of Shu *et al.* (27) with respiratory inhibitors suggested that cytochrome oxidase participates in uredospore respiration. The object of the present study was to elucidate the nature and site of the terminal oxidase and the electron transport system in the rust spore. It also seemed desirable to look for similarities between the route of respiration in the stem rust and that of other fungi and higher plants.

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Materials and Methods

Uredospores were collected from Stewart wheat plants inoculated with *Puccinia graminis* var. *tritici*, Race 15B. Spores of Race 56 were kindly supplied by Dr. D. R. Knott of the University of Saskatchewan. The spores were screened and appeared free of other fungal contaminants. Fresh spores were used in the enzyme studies and in most of the respiration experiments.

Respiratory measurements were made at 25° or 30° C by standard Warburg technique. Alkali (20% KOH) was placed in the center well. The commercial N_2 was scrubbed with alkaline pyrogallol to remove traces of O_2. Aluminum foil wrapped around vessels flushed with CO/O_2 served to exclude light. Two lamps (150-watt G.E. photoflood), delivering from 300 to 350 ft-c of white light at the bottom of the vessels, were used for reversal of CO-inhibition.

Spores were seeded evenly on solutions of coumarin (100 μg/ml) and allowed to germinate in covered glass dishes. Coumarin promoted germination (11) and thick, buff-colored mats of mycelium were observed in 24 hours or less. The mycelium was cut into sections, blotted dry, rinsed in cold buffer or medium for grinding, and blotted again. Microscopic examination showed negligible bacterial contamination after washing of the material. The germinated spores were weighed after drying for 24 hours at 85° C.

In the measurement of respiratory affinity for O_2, spore material was treated as follows: ungerminated spores were floated on 1.5 ml of 1×10^{-4} *M* pelargonaldehyde solution to stimulate respiration (11). Mats of germinated spores were freed of excess liquid and were placed in the bottom of dry 5-ml vessels. The average dry weight per vessel of germinated spores was 32.7 mg. A water-soaked paper wick in the side arm gave a moist gas phase. Warburg vessels were gassed with N_2/O_2 mixtures of approximately 1 to 2% O_2. The manometric system was closed after flushing with gas and was equilibrated for 5 minutes at 30° C. The pressure was then released at zero time, the system reclosed, and readings taken every 5 minutes until the available O_2 was consumed. The amount of O_2 remaining and the rate of O_2 uptake were calculated for each time interval.

Particulate enzymes were extracted from ungerminated and germinated spores at 0° C using a Ten Broeck homogenizer and 80–120 mesh silica. The breakage of spores was virtually complete. In the routine extraction procedure, 200 mg of fresh spores or 185 mg (dry weight) of germinated spores was ground several times with about 1 g of silica and 4 ml of cold grinding medium. The latter consisted of 0.4 *M* sucrose (pH 7.4), 0.01 *M* tris (hydroxymethyl)-aminomethane (Tris), 0.01 *M* KH_2PO_4–K_2HPO_4, 0.001 *M* ethylenediamine tetraacetate (disodium EDTA), and 0.0001 *M* cysteine. The cell debris, etc., was removed by centrifuging at 1000*g* at 2° C for 3 minutes. The residue was washed with grinding liquid and centrifuged. The supernatants were combined and centrifuged at 20,000*g* at 2° C for 20 minutes to sediment a yellowish-tinged pellet containing the respiratory enzymes. The pellet (referred to as particles) was homogenized into 4 to 8 ml of cold 0.4 *M* sucrose (pH 7.4) in 0.01 *M* Tris-phosphate buffer. Protein contents (23) of these preparations averaged 1.13 mg/ml for ungerminated spores and 0.85 mg/ml for germinated spores. When spore material was ground with digitonin medium (1% in pH 7.4

0.4 *M* sucrose, 0.01 *M* phosphate), the debris was separated, then reground, and kept at 0° C for one-half hour to aid in particle disintegration (16, 17).

Particulate enzymes were assayed at 25° C with a Beckman DU spectrophotometer. The blank contained particles plus buffer. The activities of cytochrome oxidase, succinic-cytochrome *c* reductase, and DPNH- and TPNH-cytochrome *c* reductase were followed at 550 mμ. Cytochrome *c* was reduced with a few crystals of sodium hydrosulphite. Malic, isocitric, and glycerol-1-phosphate (GP) dehydrogenases were assayed by coupling their reduction of DPN to the reduction of cytochrome *c* at 550 mμ. Dye reductase was measured at 340 mμ in the presence of DPNH or TPNH and 2,6-dichlorophenol-indophenol (DCIP).

Mammalian cytochrome *c* (Sigma) was assayed before use (8). Eastern Chemicals Corporation supplied sodium DL-glycerol phosphate (98% α-form). Antimycin A was obtained from the Wisconsin Alumni Research Foundation. Fresh solutions of Antimycin A, SN 5949 (2-hydroxy-3(2-methyloctyl)-1,4-naphthoquinone), menadione (2-methyl-1,4-naphthoquinone), HOQNO (2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide), vitamin K_1, and *dl*-α-tocopheryl acetate were prepared in ethanol. Suitable controls with alcohol were included.

Results

Effect of Inhibitors on the Respiration of Germinated and Ungerminated Uredospores

Table I shows the effect of a number of enzyme inhibitors on the respiration of ungerminated spores. Maximum inhibition or stimulation was observed in 120 or 180 minutes. A pronounced inhibition was obtained with cyanide, azide, and CO-dark, which block cytochrome oxidase (3, 8, 26, 28). The effects of cyanide disappeared with time. The initial inhibition of respiration by CO was reversed 90% in light. Diethyldithiocarbamate and particularly *p*-nitrophenol stimulated oxygen uptake at 0.001 *M* or less. *o*-Nitrophenol was inactive (13). Respiration was almost eliminated with low concentrations (10 to 80 μg/ml) of Antimycin A and SN 5949 but was more resistant to HOQNO. These substances interfere with the transfer of electrons between cytochrome *b* and *c* (5, 16, 19, 37).

Table II gives the response of the respiration of germinated spores to these same inhibitors. The over-all pattern of inhibition and stimulation of respiration appeared similar to that for ungerminated spores. However, both cyanide and azide were less inhibitory and diethyldithiocarbamate failed to stimulate. The rather weak inhibition by Antimycin A and HOQNO may reflect a poor penetration of these compounds. The results with SN 5949 are incomplete and are not provided. Light was relatively ineffective in reversing the inhibition of germinated spores by CO-dark. Mats of mycelium tended to "clump" in the flasks, likely reducing the entrance of light into the tissue.

Other inhibitors of metallo-oxidases tested on the respiration of germinated and ungerminated spores included 8-hydroxyquinoline, phenylthiourea, ethyl xanthate, 2,3-dimercaptopropanol (BAL), and *p*-chloromercuribenzoate (PCMB). Respiration was either stimulated (BAL and PCMB) or weakly inhibited at concentrations strongly inhibitory to copper and sulphydryl

TABLE I

Inhibitor effects on the respiration of ungerminated uredospores*

Inhibitor	Concentration	% Inhibition†	
		Initial	120 minutes
Azide	1×10^{-2} *M*	91	84
	1×10^{-3} *M*	82	68
	1×10^{-4} *M*	50	14
Cyanide	1×10^{-3} *M*	91	0
	1×10^{-4} *M*	82	0
	1×10^{-5} *M*	39	0
CO/O_2-dark	95% CO/5% O_2	85 (12)	75 (8)
CO/O_2-light	95% CO/5% O_2	8 (7)	16 (7)
p-Nitrophenol	1×10^{-3} *M*	+13	+ 25
	5×10^{-4} *M*	+33	+111
	1×10^{-4} *M*	+55	+130
	1×10^{-5} *M*	+18	+ 37
Sodium diethyldithio-carbamate	1×10^{-2} *M*	7	63
	1×10^{-3} *M*	+36	+ 20
	5×10^{-4} *M*	+ 8	+ 4
Antimycin A	80 μg	90	96
	20 μg	77	90
	10 μg	60	87
HOQNO	60 μg	15	33
	20 μg	22	57
	10 μg	17	53
SN 5949	40 μg	47	77 (180 minutes)
	20 μg	58	89 (180 minutes)
	10 μg	46	79 (180 minutes)
	6 μg	38	67 (180 minutes)

*Vessels contained 10 to 25 mg spores, 0.03 *M* phosphate (pH 6.4) in a total volume of 1.5 ml at 30° C.
†Control rate equals 100%. Figures in parentheses indicate number of vessels averaged. Plus sign shows stimulation.

enzymes. Repeated experiments with crude and acetone-fractionated spore extracts failed to reveal polyphenol oxidase or quinone reductase as previously reported from this laboratory (10). Chlorogenic acid and catechol were tried as substrates in tests for polyphenol oxidase while the sensitive method of Wosilait and Nason (38) was used in attempts to detect quinone reductase.

Oxygen Affinity of Spore Respiration

One technique which has had recent success in the differentiation of terminal oxidases at the tissue level has been the estimation of O_2 affinity (30). Figure 1 shows a double reciprocal plot (22) of the data obtained for a representative experiment in which the O_2 affinity of respiring uredospores was measured. The spores were saturated with O_2 at the start of the readings since controls in pure O_2 respired at an equal rate. The uptake of gas in the controls was essentially linear during the reaction period. While some deviation from the expected kinetics occurred at the initial levels of O_2, a first-order dependence

TABLE II

Inhibitor effects on the respiration of germinated uredospores*

Inhibitor	Concentration	% Inhibition†	
		Initial	120 minutes
Azide	1×10^{-2} *M*	73	92
	1×10^{-3} *M*	35	64
	5×10^{-4} *M*	13	60
	1×10^{-4} *M*	3	3
Cyanide	1×10^{-3} *M*	12	52
	5×10^{-4} *M*	7	48
	1×10^{-4} *M*	28	57
	1×10^{-5} *M*	8	8
CO/O_2-dark	95% CO/5% O_2	62 (13)	61 (12)
CO/O_2-light	95% CO/5% O_2	42 (6)	55 (5)
p-Nitrophenol	5×10^{-3} *M*	+ 27	29
	1×10^{-3} *M*	+ 61	+ 9
	5×10^{-4} *M*	+110	+ 88
	1×10^{-4} *M*	+141	+137
	5×10^{-5} *M*	+ 54	+ 64
Sodium diethyldithiocarbamate	1×10^{-2} *M*	86	86
	1×10^{-3} *M*	4	31
	5×10^{-4} *M*	3	3
Antimycin A	25 μg	34	34
	15 μg	51	50
	10 μg	20	42
	5 μg	17	0
HOQNO	40 μg	16	16
	20 μg	36	26
	5 μg	17	13

*Control vessels with air had an average of 13.8 mg dry weight of 24-hour-old germinated spores, 0.03 *M* phosphate buffer (pH 6.4) in a total volume of 0.75 ml. Controls for CO/O_2 experiments contained N_2/O_2 (95:5) as the gas phase and an average of 19.8 mg dry weight per vessel. Average initial Q_{O_2} of controls: 3.1 (air) and 2.14 (N_2/O_2).
†Control rate equals 100%. Figures in parentheses indicate number of vessels averaged. Stimulation is shown by plus sign.

on O_2 was noted at concentrations of less than 1%. In seven experiments, the average O_2 affinity of germinated spores was 0.008 atm and for four experiments with ungerminated spores, it was 0.0067 atm. These figures refer to the pressure of O_2 which allows half the maximum rate of respiration (the K_m).

Cytochrome Oxidase and Associated Particulate Enzymes

Activity of cytochrome oxidase could be shown in cell-free fractions prepared in digitonin or sucrose–phosphate medium. The results of these experiments are presented in Figs. 2 to 4. Figure 2 illustrates the time course of oxidation of reduced cytochrome *c* by a digitonin extract from ungerminated spores. The oxidase was saturated with cytochrome *c* under the conditions of assay, yet it was estimated that only 70% of the calculated maximum velocity was attained (12).

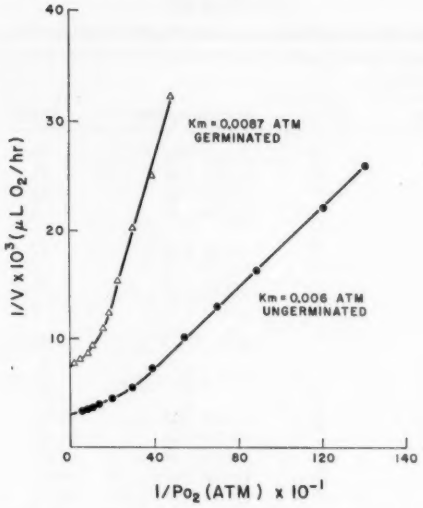

FIG. 1. Lineweaver–Burk plot of the effect of oxygen tension on spore respiration. Contents of vessels as described in the section Materials and Methods.

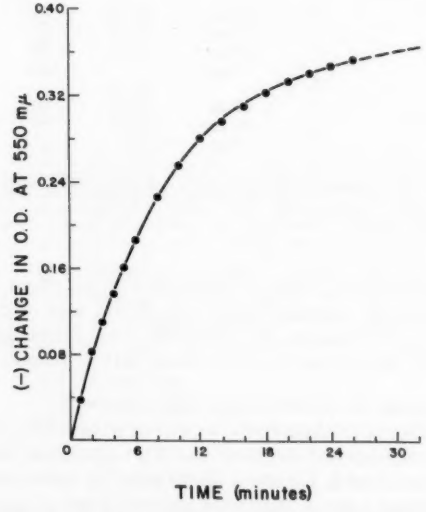

FIG. 2. Time course of cytochrome oxidase activity by a digitonin preparation from ungerminated spores. The cuvette contained 3.2×10^{-5} *M* ferrocytochrome *c*, 0.4 *M* sucrose, 0.01 *M* phosphate buffer (pH 7.4), 0.133% digitonin, and 0.134 mg of particle protein in a total volume of 3.0 ml.

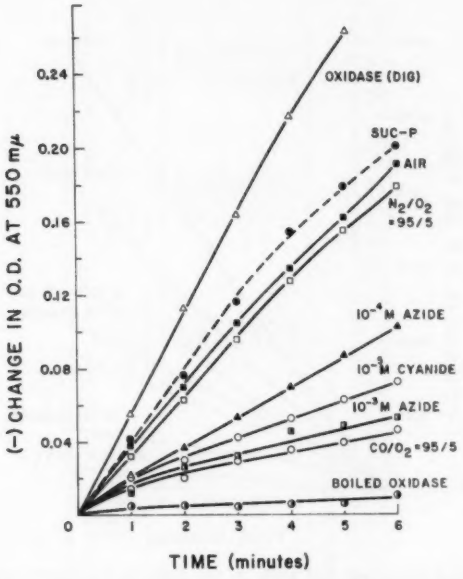

FIG. 3. Effect of cyanide, azide, and CO on the digitonin-extracted cytochrome oxidase from ungerminated spores. Reaction cuvettes containing KCN or NaN_3 had 3.2×10^{-5} *M* ferrocytochrome *c*, 0.4 *M* sucrose, 0.01 *M* phosphate buffer (pH 7.4), 0.10% digitonin, and 0.156 mg particle protein per total volume of 3.0 ml. Cuvettes flushed with gas mixtures and the corresponding control in air contained the same except for a different extract (0.189 mg protein/3.0 ml). The cuvette with sucrose–phosphate (Suc-P) particles contained cytochrome *c*, sucrose, and phosphate as before and 0.854 mg protein. Control: boiled particles, cytochrome *c*, sucrose, and phosphate buffer.

The cytochrome oxidase was localized in a particulate fraction, presumably mitochondrial (see Table VI). The activity curves of cytochrome oxidase extracted from ungerminated and germinated spores of Race 15B can be seen in Figs. 3 and 4. Controls minus particles or with boiled particles showed a negligible oxidation of ferrocytochrome *c*. Enzyme activity was approximately proportional to the concentration of particles. The oxidase was stable for several hours at 0° C. The dotted line in Fig. 3 represents particles prepared in sucrose–phosphate medium while the other curves are for digitonin extracts. Both types of preparations behaved similarly towards inhibitors. A high concentration of phosphate (0.1 *M*) severely inhibited the enzyme. The oxidase was completely inhibited by 0.001 *M* cyanide (not shown) and inhibited about 80% with 1×10^{-5} *M* cyanide. Azide was somewhat less effective. Carbon monoxide/O_2 in the dark reduced the activity to 18% that of a N_2/O_2 (95:5) control. This inhibition was abolished by light. The participation of cytochrome *c* peroxidase seems doubtful considering the response of the enzyme to CO. The enzyme was insensitive to SN 5949 and to Antimycin A.

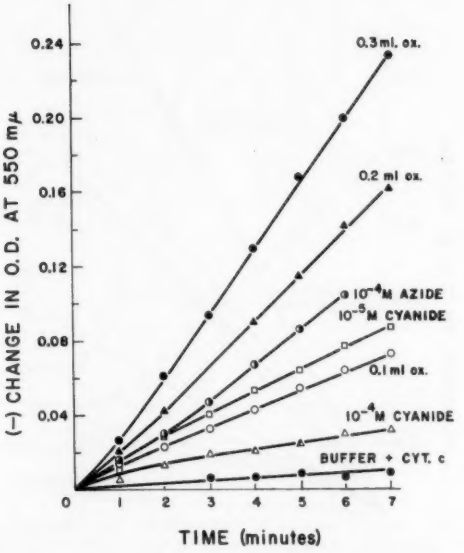

FIG. 4. The effect of cyanide and azide on the digitonin-extracted cytochrome oxidase (ox.) from germinated spores. The cuvette contained cytochrome *c*, sucrose, phosphate buffer, digitonin, and KCN or NaN_3 as in Fig. 3 plus 0.147 mg of particle protein (0.3 ml ox.) in a total volume of 3.0 ml. Control: minus particles.

Since it appeared that cytochrome oxidase was mediating the respiration of the spore, it became of interest to assay quantitatively the capacity of this enzyme in cell-free particulates. In the experiments listed in Table III, a known amount of spore material was thoroughly extracted in sucrose–phosphate or digitonin medium. The initial rates of ferrocytochrome *c* oxidation by such extracts were converted to the equivalent amount of O_2 reduced per hour per gram of material. Respiration measurements were made with the same samples of spores.

The inclusion of 1% digitonin in the extraction solution gave, on the average, a fivefold increase in cytochrome oxidase activity over that in the sucrose–phosphate extract (Table III). Rust spore particles required mechanical disruption in the digitonin to disperse the oxidase and to obtain a higher apparent yield. The respiration recovered in extracts of ungerminated spores (experiment 2) was only 9.6% of that in the intact spore.

The results with germinated spores were more encouraging and it proved possible to recover about 67% of the total respiration (experiments 6 and 7). Actually, the yield of cytochrome oxidase did not increase substantially since the respiratory rate of germinated spores fell by about one-fifth. (The O_2 uptake by fresh spores is approximately equivalent on a fresh or a dry weight basis.)

TABLE III

Quantitative determination of cytochrome oxidase and respiration in germinated and ungerminated uredospores

Experiment*	Spores	Extraction medium†	Protein/ml, mg	μMoles cyt. *c* oxidized/ minute/g‡	μLiters O_2 reduced/ hour/g§	Respiration, μliter O_2/ hour/g‖	% Respiration recovered
1	Ungerm. 15B	Standard	0.880	0.765 (2)	257	11,900	2.2
2	Ungerm. 15B	Digitonin	0.647	2.67 (4)	893	8,550	9.6
3	Germ. 56	Standard	0.853	1.19 (3)	399	2,950	13.5
4	Germ. 56	Standard	1.09	1.36 (3)	457	3,860	11.8
5	Germ. 15B	Standard	1.12	1.39	465	2,320	20.0
6	Germ. 15B	Digitonin	0.49	2.52	844	1,235	68.4
7	Germ. 56	Digitonin	0.950	5.06	1700	2,615	65.0

*Race 56 and Race 15B spores were germinated for 24 hours in experiments 3 to 6 and for 48 hours in experiment 7.

†Composition given in the section Materials and Methods.

‡Figures in parentheses show number of experiments averaged. Based on per gram fresh weight of ungerminated and per gram dry weight of germinated spores. Reaction cuvettes in the standard runs contained 3.2×10^{-5} *M* ferrocytochrome *c*, 0.4 *M* sucrose, 0.01 *M* Tris-phosphate buffer (pH 7.4), and an average of 0.327 mg protein in a final volume of 3.0 ml. Cuvettes in the digitonin runs contained cytochrome *c*, sucrose, and phospate as above, plus 0.07% digitonin and an average of 0.160 mg protein per 3.0 ml.

§Calculated from μmoles of cytochrome *c* oxidized/minute/g.

‖Respiration measured at 25° C in 0.03 *M* phosphate buffer (pH 6.4).

As shown in Fig. 5, particles isolated from germinated and ungerminated uredospores can catalyze the reduction of cytochrome *c* with succinate as substrate. Particles without substrate did not reduce cytochrome *c* faster than the control with succinate and no added enzyme. Disruption of the spore particles with digitonin caused a complete loss of activity, suggesting some degree of spatial organization of the enzyme. The stability of the enzyme varied widely among preparations, particularly those from ungerminated spores. Most of the activity had disappeared inside of 1 hour at 0° C. The oxidation of succinate was typically inhibited by malonic acid (Fig. 5 and Table IV).

A rapid increase in optical density at 550 mμ occurred upon the addition of particles to ferricytochrome *c* in the presence of DPNH and 0.001 *M* cyanide (Fig. 5). Ferricytochrome *c* was slowly reduced in the absence of particles. Experiments showed that TPNH was about 16% as active as DPNH in the system. Digitonin extraction of the particles eliminated the activity. The enzyme in particles from germinated spores usually lost its activity within 1 hour at 0° C.

Other enzymes localized in the particulate fraction included malic dehydrogenase, GP dehydrogenase, and isocitric dehydrogenase. An increase in optical

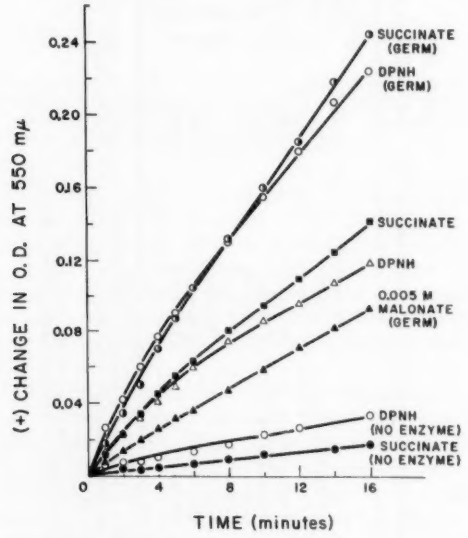

FIG. 5. Succinic-cytochrome *c* reductase and DPNH-cytochrome *c* reductase activities in particles from germinated (germ) and ungerminated spores. Reaction cuvettes contained 3.2×10^{-5} *M* ferricytochrome *c*, 0.4 *M* sucrose, 0.01 *M* Tris-phosphate buffer (pH 7.4), 0.001 *M* KCN, either 8.7×10^{-5} *M* DPNH or 0.015 *M* succinate in a final volume of 3.0 ml. Malonate was used in the presence of 0.015 *M* succinate. Particle protein per cuvette (mg): 0.354 (germinated), 0.318 (ungerminated). Closed triangle: succinic-cytochrome *c* reductase plus 0.005 *M* malonic acid. Controls: DPNH or succinate minus particles.

TABLE IV

Effect of certain inhibitors and stimulators on the activity of the particulate enzymes

Enzyme(s)	Inhibitor or stimulator	Concentration†	% Inhibition (−) or stimulation (+)‡	
			Germinated	Ungerminated
Fresh particles:	HOQNO	10 μg	−77 (−59 to −85)(4)	—
DPNH-cytochrome	SN 5949	10 μg	−86 (−82 to −90)(4)	—
c reductase	SN 5949	3 μg	—	−40
	Antimycin A	10 μg	−86 (−85 to −86)(2)	—
	Menadione	5×10^{-6} *M*	+240	—
	Vitamin K_1	5×10^{-6} *M*	− 4	—
Aged particles:*	Antimycin A	10 μg	− 10	—
DPNH-cytochrome	SN 5949	10 μg	− 4	—
c reductase	Menadione	2.5×10^{-6} *M*	—	+37
Fresh particles:	HOQNO	10 μg	−43 (−25 to −60)(2)	—
Succinic-cytochrome	SN 5949	10 μg	−45 (−15 to −63)(3)	—
c reductase	SN 5949	3 μg	− 44	−54 (−29 to −83)(3)
	Antimycin A	5 μg	—	−43
	Menadione	5×10^{-6} *M*	+232	—
	Malonate	1×10^{-2} *M*	—	−61
		5×10^{-3} *M*	− 68	—
		1.7×10^{-3} *M*	− 44	—
Cytochrome oxidase	SN 5949	10 μg	+ 2	—
Dye reductase	Menadione	5×10^{-6} *M*	+ 10	—
α-GP-dehydrogenase	DPN	3×10^{-4} *M*	+ 84	—

*Particles held at 0° C for 45 minutes.
†Average particle protein in 3.0 ml reaction volume was 0.292 mg (germinated) and 0.311 mg (ungerminated).
‡Per cent of suitable control rate. Figures in parentheses give range observed and number of averaged experiments, respectively

density at 550 mμ was observed when particles were added to cuvettes containing substrate, DPN, ferricytochrome *c*, and 0.001 *M* cyanide. In Fig. 6, it is seen that particles from germinated spores oxidized malate and GP while particles from ungerminated spores oxidized malate and isocitrate. The soluble supernatant failed to reduce DPN when any of the substrates was added. The oxidation of malate, GP, and isocitrate could be coupled to the reduction of either DPN or DCIP (645 mμ). The dehydrogenases were inactive with TPN. Table IV shows that the addition of DPN to particles from germinated spores caused an increase of 84% in the rate of ferricytochrome *c* reduction using GP as substrate.

Cytochrome oxidase prepared and assayed in a medium containing 0.01 *M* Tris buffer was inactivated before the available ferrocytochrome *c* had been depleted (Fig. 6).

The demonstration that cell-free particles could oxidize malic, isocitric, and succinic acids was suggestive of a citric acid cycle. Preliminary experiments (manometric) showed that particles from germinated spores could oxidize slowly citric, α-ketoglutaric, isocitric, malic, and succinic acids when fortified with cocarboxylase, DPN, glutathione, Coenzyme A, cytochrome *c*, $MgCl_2$, ADP, and ATP.

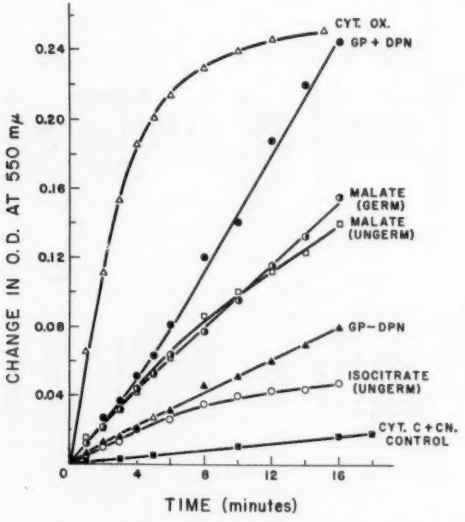

FIG. 6. Demonstration of several DPN-linked cytochrome *c* reductases in particles from germinated and ungerminated spores. The reaction cuvettes contained 3.2×10^{-5} *M* ferricytochrome *c*, 3×10^{-4} *M* DPN, 0.4 *M* sucrose, 0.01 *M* Tris-phosphate buffer (pH 7.4), 0.001 *M* KCN, and either 0.02 *M* DL-glycerolphosphate (GP) or 0.01 *M* isocitrate in a final volume of 3.0 ml. Particle protein per cuvette (mg): 0.320 (GP) and 0.306 (isocitrate). Malic-cytochrome *c* reductase assayed as above except the cuvettes contained 0.005 *M* malate, Tris-phosphate buffer (pH 8.0), and 0.306 mg of particle protein. The cuvette for the cytochrome oxidase reaction (open triangle) had 3.2×10^{-5} *M* ferrocytochrome *c*, 0.4 *M* sucrose, 0.01 *M* Tris-phosphate buffer (pH 7.4), and 0.354 mg of particle protein per 3.0 ml. Control: minus particles.

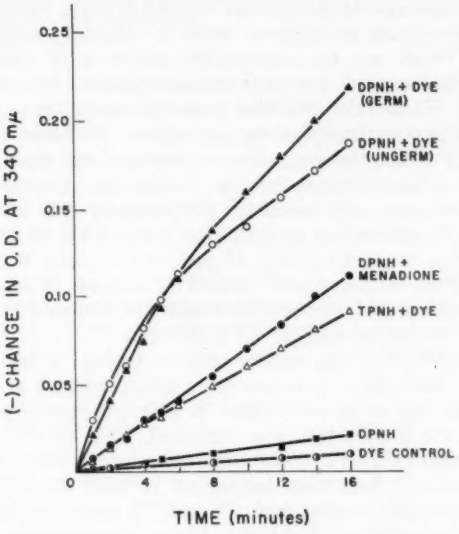

FIG. 7. The activity of dye reductase in particles from ungerminated and germinated spores. The reaction cuvettes contained 5.8×10^{-5} *M* DPNH, 2.5×10^{-5} *M* 2,6-dichlorophenol-indophenol, 0.038 *M* sucrose, 0.0095 *M* Tris-phosphate buffer (pH 7.4), 0.001 *M* KCN in a total volume of 3.0 ml. Particle protein per cuvette (mg): 0.306 (ungerminated), 0.256 (germinated). Open triangles: 6.8×10^{-5} *M* TPNH and 0.256 mg of particle protein (germinated) per 3.0 ml of reaction volume. Closed circles: 5×10^{-5} *M* menadione and 0.256 mg of particle protein (germinated). Closed squares: 8.7×10^{-5} *M* DPNH, 0.4 *M* sucrose, 0.01 *M* Tris-phosphate buffer (pH 7.4), and 0.342 mg of particle protein (germinated) per 3.0 ml. Dye control: cuvette contents as for dye reductase except no particles added.

An active dye reductase was found in spore particles as shown in Fig. 7. The visible blue color of the dye disappeared within 16 minutes after the addition of enzyme. Negligible rates of DPNH and TPNH oxidation occurred when particles were omitted. Reduced DCIP was not reoxidized in the absence of cyanide suggesting that the endogenous cytochrome *c* was removed during particle isolation. The enzyme was quite stable at 0° C. Dispersion of the particles with digitonin did not increase the amount of activity. Indophenol dye was a better electron acceptor than either ferricyanide or menadione. The rate of dye reduction with TPNH as the hydrogen donor was 10% of that with DPNH.

Effects of Inhibitors on the Particulate Enzymes

Table IV gives the response of the particulate enzymes to several of the compounds tested on spore respiration (Tables I and II). The DPNH-cytochrome *c* reductase in fresh particles from germinated spores was strongly inhibited by Antimycin A, SN 5949, and HOQNO. About 20% of the activity proved to be insensitive. The DPNH-cytochrome *c* reductase in particles aged for 45 minutes at 0° C was only 20% as active as in the fresh preparation and

was relatively insensitive to Antimycin A and SN 5949. Menadione at 5×10^{-6} *M* markedly stimulated the system while its related analogue, vitamin K_1, had no effect. There was no appreciable reduction of cytochrome *c* when particles were left out of the menadione-activated system. α-Tocopheryl acetate (vitamin E) weakly inhibited the reductase. The activity of the dye reductase was slightly stimulated by menadione. Reduced TPN was almost as effective as DPNH in the menadione-stimulated reaction.

The activity of succinic-cytochrome *c* reductase in fresh particles from germinated spores was only partially inhibited by SN 5949 and HOQNO (Table IV). Inhibition values ranged from 15 to 63% in three experiments with SN 5949 at a concentration of 10 μg/3 ml. Results with HOQNO were similar. The addition of menadione caused an increase in activity. Malonate, the classical inhibitor of succinic dehydrogenase, diminished the activity by 68% at 5×10^{-3} *M* and by 44% at 1.7×10^{-3} *M*.

Considerable difficulty was experienced in trying to establish the effects of inhibitors on particulate enzymes from ungerminated spores (Table IV). This was due to the wide variability in activity among preparations. The DPNH-cytochrome *c* reductase was inhibited 40% by SN 5949 (1 μg/ml). Menadione restored DPNH-cytochrome *c* reductase activity to aged particles. Succinic-cytochrome *c* reductase responded variably to SN 5949 at a level of 1 μg/ml. Inhibitions ranging from 29 to 83% were recorded in these runs. Single experiments indicated that this reductase was sensitive to Antimycin A and to malonate. The dye reductase was not inhibited by SN 5949. Menadione had no effect on cytochrome oxidase.

Comparison of Enzyme Activities

A comparison of enzyme rates in spore particulates showed that germination elicits a significant increase in the activity of several enzymes. Table V presents the computed averages and standard deviations of the results. The rates of DPNH oxidation were corrected for dye reduction. A test of significance at a probability level of 5% or less showed that the activities of cytochrome oxidase, succinic-cytochrome *c* reductase, and DPNH-cytochrome *c* reductase were higher in germinated than in ungerminated spores. The activities of dye reductase and malic dehydrogenase were not significantly

TABLE V

Relative rates of enzyme activities in particulate fractions from germinated and ungerminated uredospores

Enzyme(s)	Substrate assayed	μMoles×10²/minute/mg protein*	
		Germinated	Ungerminated
Cytochrome *c* oxidase	Cyt. *c*	3.99±1.21 (8)	2.23 ±0.819 (5)
DPNH-cytochrome *c* reductase	Cyt. *c*	1.92±1.52 (11)	0.732±0.282 (10)
Succinic-cytochrome *c* reductase	Cyt. *c*	1.64±1.21 (10)	0.504±0.038 (9)
DPNH-dye reductase	DPNH	2.70±0.952 (11)	1.68 ±1.16 (6)
Malic-cytochrome *c* reductase	Cyt. *c*	1.08±0.788 (6)	0.519±0.353 (6)
α-GP-cytochrome *c* reductase	Cyt. *c*	0.281	—

*Figures in parentheses show the number of experiments averaged. One milligram particle protein represents 33.8 mg dry weight of 24-hour-old germinated spores and 22.4 mg fresh weight of ungerminated spores.

different. Cytochrome oxidase was more active than each of the other enzymes with the exception of dye reductase.

Soluble System

The supernatant remaining after fractionation of particles from germinated spores catalyzed a rapid oxidation of DPNH with added DCIP or cytochrome *c*. Oxygen could not replace the dye or cytochrome *c*. The supernatant also catalyzed the reduction of cytochrome *c* without added DPNH. Table VI shows the results obtained with fresh and boiled extract. A soluble dye reductase was indicated since boiling decreased the rate of DCIP reduction. About 50% of the total dye reductase activity was in this fraction.

TABLE VI

Enzymatic and nonenzymatic activities of the supernatant from ungerminated spores

System assayed*	μMoles/minute/g fresh weight	
	Fresh	Boiled
DPNH-cytochrome *c* reductase	0.765	0.450
DPNH-dye reductase	0.760	0.084
DPNH oxidase	0.0	0.0
Cytochrome *c* reduced	0.482	0.393
Cytochrome *c* oxidase	0.084	0.084
Succinic-cytochrome *c* reductase	0.566	0.425
Malic-DPN-cytochrome *c* reductase	0.710	0.392

*Oxidation of DPNH, where present, was measured at 340 mμ. Otherwise cytochrome *c* oxidation or reduction was assayed at 550 mμ.

Discussion

The results of these experiments indicate that electron transport in the uredospore is catalyzed by a series of carriers like those of other fungi (6, 21, 26, 28) and of higher plants (16). That is, the oxidation of cell metabolite is coupled to the sequential reduction of pyridine nucleotide, flavoprotein, cytochrome, and then oxygen. Inhibition of uredospore respiration by CO and its relief by light (Tables I and II) is direct evidence for a functional cytochrome oxidase. The response of other fungi to CO is similar (3, 26, 28). Further support for an active cytochrome system was the strong retardation of respiration by Antimycin A and SN 5949. Also, the O_2 affinity of spore respiration falls within the range of O_2 affinities observed for other cytochrome-dependent tissues. For example, respiring slices of Aroid spadix and potato tuber have a K_m of 0.002 atm of O_2 (33, 39), and pea internodes, 0.031 atm of O_2 (9). Mycelium of the fungus *Myrothecium verrucaria* has an affinity of less than 0.005 atm of O_2 (36). The high affinity for O_2 of uredospores definitely rules out ascorbic acid oxidase or polyphenol oxidase as respiratory carriers (39). For sake of comparison, a crude polyphenol oxidase was prepared from sweet potatoes. The enzyme had an affinity of 0.035 atm of O_2. This is one-fifth the value found for uredospore respiration.

It was not too surprising to find that only a small fraction of the cytochrome oxidase could be extracted from the uredospore (Table III). This enzyme is notably sensitive to the conditions of isolation (30). It is also likely that the

exogenous cytochrome is not as efficient in electron transfer as is endogenous cytochrome *c* (29). Assuming a loss during extraction as low as 40%, there would still be sufficient cytochrome oxidase to balance the O_2 uptake by germinated spores.

Several conclusions can be drawn from the data in Table IV. First, the inhibition of DPNH-cytochrome *c* reductase by Antimycin A, SN 5949, and HOQNO reveals a direct pathway of DPNH oxidation which is different from the Antimycin-A-insensitive pathway reported in some mitochondria (14, 16, 24, 25). The response of the enzyme to Antimycin A is like that of the DPNH-cytochrome *c* reductase in lupine mitochondria (18). It is interesting to note that about 80% of the DPNH-cytochrome *c* reductase in spore particles is labile and that this is the fraction sensitive to Antimycin A, SN 5949, and HOQNO. This labile fraction appears to account for the major share of electron transfer between DPNH and added cytochrome *c*. The reductase in particles from germinated spores aged rapidly after isolation, possibly explaining the low inhibition seen with SN 5949 (Table IV). Second, Antimycin A and both SN 5949 and HOQNO are effective inhibitors of succinate oxidation by mitochondria of higher plants (5, 14, 17, 24). This was not the case with uredospore particles in which 50% of the succinic-cytochrome *c* reductase activity did not involve an Antimycin-sensitive factor. There may be two routes of succinate oxidation in the spore particle, one of which is resistant to Antimycin A, etc., and which may be activated only when exogenous cytochrome *c* is present (16). Martin and Morton (25) have reported similar results with mitochondria from wheat roots. We were unable to test the inhibitors on the oxidation of succinate alone since cytochrome *c* was washed out of the particle during fractionation. It is probable that menadione acts as an alternative electron carrier between flavoprotein and cytochrome *c* (34).

The inability of spore particles to oxidize DPNH unless cytochrome *c* was added suggests that this haemoprotein is a component of their hydrogen transport system. The inhibition of DPNH-cytochrome *c* reductase by Antimycin A tends to suggest a classical *b*, *c*, *a*-a_3 sequence of cytochrome. Other fungi are known to have these respiratory components (4, 21, 26, 32). Low-temperature (−196° C) spectra of extracts from digitonin-treated particles as well as digitonin extracts of whole spores did not resolve any cytochrome components except a trace of a_3 (cytochrome oxidase) at 600 mμ. These extracts contained carotenoid pigments which seriously interfered with the resolution of the dilute cytochrome. Attempts to remove the pigments were not successful.

The increased activity of succinic-cytochrome *c* reductase in germinated spores (Table V) may follow the disappearance of endogenous malonate during germination (31). The general increase in activity of the respiratory enzymes after germination may reflect an activation of the citric acid cycle. Spore particles appear to have an excess of cytochrome oxidase relative to the other respiratory enzymes. Comparative findings have been made with mitochondria from cauliflower buds (7) and potato tubers (17). The over-all limiting factor in the spore particle appears to be the rate at which cytochrome *c* is reduced.

It seems unlikely that the soluble system plays a significant role in spore respiration. There is no evidence to implicate ascorbic acid in the soluble reactions as there is in potato tuber (15). The heat-resistant activity possibly involves quinones as redox intermediates (21).

Kaul and Shaw (20) have proposed that a "redox" catalyst may be generated in the tissue of rust-infected wheat. Such a catalyst may accelerate respiration and increase the contribution of the pentose phosphate pathway as does methylene blue for instance (2). Quinonic carriers may occur in rusted wheat tissue and it would seem of interest to isolate them and to test their effect on the particulate enzymes, respiration, and catabolism of glucose in the wheat leaf and the rust spore.

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References

1. Allen, P. J. 1959. Metabolic considerations of obligate parasitism. *In* Plant pathology, problems and progress, 1908–1958. University of Wisconsin Press, Madison. pp. 119–129.
2. Ap Rees, T. and Beevers, H. 1960. Pathways of glucose dissimilation in carrot slices. Plant Physiol. **35**, 830–838.
3. Boulter, D. 1957. The oxidases of *Gelasinospora tetrasperma* in relation to the endogenous respiration. J. Gen. Microbiol. **16**, 305–316.
4. Boulter, D. and Derbyshire, E. 1957. Cytochromes of fungi. J. Exptl. Botany, **8**, 313–318.
5. Chance, B. and Hackett, D. P. 1959. The electron transfer system of skunk cabbage mitochondria. Plant Physiol. **34**, 33–49.
6. Cheng, S. C. 1954. The terminal respiratory enzymes in *Neurospora tetrasperma*. Plant Physiol. **29**, 458–467.
7. Crane, F. L. 1957. Electron transport and cytochromes of sub-cellular particles from cauliflower buds. Plant Physiol. **32**, 619–625.
8. Darby, R. T. and Goddard, D. R. 1950. The effects of cytochrome oxidase inhibitors on the cytochrome oxidase and respiration of the fungus *Myrothecium verrucaria*. Physiol. Plantarum, **3**, 435–446.
9. Eichenberger, E. and Thimann, K. V. 1957. Terminal oxidases and growth in plant tissues. IV. On the terminal oxidases of etiolated pea internodes. Arch. Biochem. Biophys. **67**, 466–478.
10. Farkas, G. L. and Ledingham, G. A. 1959. Studies on the polyphenol–polyphenoloxidase system of wheat stem rust uredospores. Can. J. Microbiol. **5**, 37–46.
11. Farkas, G. L. and Ledingham, G. A. 1959. The relation of self-inhibition of germination to the oxidative metabolism of stem rust uredospores. Can. J. Microbiol. **5**, 141–151.
12. Fritz, G. and Beevers, H. 1955. Cytochrome oxidase content and respiratory rates of etiolated wheat and barley seedlings. Plant Physiol. **30**, 309–317.
13. Gaur, B. K. and Beevers, H. 1959. Respiratory and associated responses of carrot discs to substituted phenols. Plant Physiol. **34**, 427–432.
14. Hackett, D. P. 1956. Pathways of oxidation in cell-free potato fractions. Plant Physiol. **31**, 111–118.
15. Hackett, D. P. 1958. Pathways of oxidation in cell-free potato fractions. II. Properties of the soluble pyridine nucleotide oxidase system. Plant Physiol. **33**, 8–13.
16. Hackett, D. P. 1959. Respiratory mechanisms in higher plants. Ann. Rev. Plant Physiol. **10**, 113–146.

17. HACKETT, D. P., HAAS, D. W., GRIFFITHS, S. K., and NIEDERPRUEM, D. J. 1960. Studies on development of cyanide-resistant respiration in potato tuber slices. Plant Physiol. **35**, 8–19.
18. HUMPHREYS, T. E. and CONN, E. E. 1956. The oxidation of reduced diphosphopyridine nucleotide by lupine mitochondria. Arch. Biochem. Biophys. **60**, 226–243.
19. JACKSON, F. L. and LIGHTBROWN, J. W. 1958. Inhibition of dihydrocozymase-oxidase acitivity of heart-muscle preparations and of certain cell-free bacterial preparations by 2-heptyl-4-hydroxyquinoline-*N*-oxide. Biochem. J. **69**, 63–67.
20. KAUL, R. and SHAW, M. 1960. The physiology of host–parasite relations. VI. Oxidation–reduction changes in wheat leaf sap caused by rust infection. Can. J. Botany, **38**, 399–407.
21. KIKUCHI, G. and BARRON, E. S. G. 1959. Electron transport system in *Fusarium lini*. Arch. Biochem. Biophys. **84**, 96–105.
22. LINEWEAVER, H. and BURK, D. 1934. The determination of enzyme dissociation constants. J. Am. Chem. Soc. **56**, 658–666.
23. LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L., and RANDALL, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265–275.
24. MARTIN, E. M. and MORTON, R. K. 1956. Enzymic properties of microsomes and mitochondria from silver beet. Biochem. J. **62**, 696–704.
25. MARTIN, E. M. and MORTON, R. K. 1956. Enzymic and chemical properties of cytoplasmic particles from wheat roots. Biochem. J. **64**, 687–693.
26. NIEDERPRUEM, D. J. and HACKETT, D. P. 1961. Cytochrome system in *Schizophyllum commune*. Plant Physiol. **36**, 79–84.
27. SHU, P., TANNER, K. G., and LEDINGHAM, G. A. 1954. Studies on the respiration of resting and germinating uredospores of wheat stem rust. Can. J. Botany, **32**, 16–23.
28. SIH, C. J., HAMILTON, P. B., and KNIGHT, S. G. 1958. Some properties of the cytochrome oxidase of *Penicillium chrysogenum*. J. Bacteriol. **75**, 623–628.
29. SLATER, E. C. 1950. The dihydrocozymase-cytochrome *c* reductase activity of heart-muscle preparation. Biochem. J. **46**, 499–503.
30. SMITH, L. and CHANCE, B. 1958. Cytochromes in plants. Ann. Rev. Plant Physiol. **9**, 449–482.
31. STAPLES, R. C. 1957. The organic acid composition and succinoxidase activity of the uredospore of the leaf and stem rust fungi. Contribs. Boyce Thompson Inst. **19**, 19–31.
32. TAKAYOSHI, H. 1955. Biochemical study of wood-rotting fungi. IV. Cytochrome oxidase of wood-rotting fungi. J. Japan. Forestry Soc. **37**, 147–152.
33. THIMANN, K. V., YOCUM, C. S., and HACKETT, D. P. 1954. Terminal oxidases and growth in plant tissues. III. Terminal oxidation in potato tuber tissue. Arch. Biochem. Biophys. **53**, 239–257.
34. WEBER, M. M., BRODIE, A. F., and MERSELIS, J. E. 1958. Possible role for vitamin K in electron transport. Science, **128**, 896–898.
35. WESSELS, J. G. H. 1959. The breakdown of carbohydrate by *Schizophyllum commune* Fr. The operation of the TCA cycle. Acta Botan. Neerl. **8**, 497–505.
36. WHITE, G. A. 1959. Atypical ascorbic acid oxidase of *Myrothecium verrucaria*. Ph.D. Thesis, Iowa State University, Ames, Iowa.
37. WISKICH, J. T., MORTON, R. K., and ROBERTSON, R. N. 1960. The respiratory chain of beetroot mitochondria. Australian J. Biol. Sci. **13**, 109–122.
38. WOSILAIT, W. D., and NASON, A. 1954. Pyridine nucleotide-quinone reductase. I. Purification and properties of the enzyme from pea seeds. J. Biol. Chem. **206**, 255–270.
39. YOCUM, C. S., and HACKETT, D. P. 1957. Participation of cytochromes in the respiration of the Aroid spadix. Plant Physiol. **32**, 186–191.

CULTURAL STUDIES OF BOLETACEAE

GYRODON MERULIOIDES AND FOUR SPECIES OF BOLETINUS[1]

MARIA E. PANTIDOU

Abstract

Methods and techniques for culturing and studying species of Boletaceae in culture are described. Some preliminary views as to the possibilities and limitations of the taxonomic criteria, to be used for identifying species in culture, are discussed. Descriptions and illustrations of *Gyrodon merulioides* (Schw.) Sing., *Boletinus cavipes* (Opat.) Kalchbr., *Boletinus pictus* (Peck) Peck, *Boletinus spectabilis* (Peck) Murrill, and *Boletinus grisellus* Peck, which have not been previously described in culture, are presented.

Introduction

Several species of Boletaceae have great potential importance in reforestation because of their mycorhizal associations with forest trees. Inoculum consisting of forest soil containing mycorhizal fungi, sporophores, or even pure cultures, is being applied in newly established forest tree nurseries in some countries. The effectiveness of this program is hampered by the uncertainty that often exists concerning the identity of the mycorhizal fungi.

Many species of Boletaceae are more or less specific to their mycorhizal host. However, field observations may be misleading when based on the presence of fruit bodies near certain trees. Under laboratory conditions evidence has been obtained of mycorhizal unions between a given species and one or more hosts, but these results are sometimes contradictory and do not appear to be applicable to forest conditions in many cases. The identification of fungi isolated directly from the ectotrophic mantles, of forest tree roots, although appearing to be a more precise method, has had limited application because descriptions of Boletaceae in culture are either inadequate or unavailable.

The work reported in this paper began in 1957. Fifty species, representing 11 genera, have been obtained in culture. Preliminary studies have shown that some species can be readily recognized from their macroscopic appearance, but that the majority are not strikingly different. It is believed, however, that a combination of macroscopic, microscopic, physiologic, and chemical characteristics would lead to the identification of many species in culture and a large culture collection would provide material for comparison with unknown cultures. A method of identifying Boletaceae from their cultural characteristics would help in the problem of host specificity and also would contribute to the knowledge of the taxonomy of the group. It is apparent that cultural characteristics often distinguish species or subspecies which are very similar in the morphology of their fruit bodies. Such instances will be discussed in the descriptions of the individual species.

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Materials and Methods

Descriptions of cultures of Boletaceae in the literature are few. Melin (1922*a*, 1922*b*, 1923*a*, 1923*b*) was the first to apply the tissue culture method in species of Boletaceae. He presented good descriptions of some species and used the cultures mainly in experiments dealing with the specificity and nature of the mycorhizal associations. Subsequent workers (How 1940; Khudyakov and Voznyakouskaya 1951; Mikola 1955; Modess 1941; Sobotka 1954, 1955) obtained a few more species of Boletaceae in culture and used them in similar studies.

The cultures were obtained from fresh fruit bodies. Tissue from old sporophores grew well but young sporophores were more suitable because they were less infested with fungi or bacteria. Growth was better in tissue taken from fruit bodies in which slight drying-out had occurred. It was noted that tissue from fruit bodies kept in the refrigerator for 3 days and from fruit bodies sent by mail and in transit for up to 40 hours grew well in culture. It should also be noted that growth rarely occurred from a piece of tissue placed flat on the agar, whereas tissue placed so that half was immersed in the agar and half projected into the air usually yielded good growth. Almost invariably the growth started from the part of the tissue above the agar.

The sporophore was broken open, and flamed fine tweezers were used to cut and transfer a portion of tissue usually taken from between the union of the pileus and stipe. The first visible growth appeared in 2 to 5 days, partly depending on the species cultured. The species of *Leccinum* were the slowest in initiating growth of the cultured tissue.

The Hagem medium, as modified by Modess (1941), was used throughout this study. It proved satisfactory for growth of most species. The cultures were grown at room temperature, which varied between 20° and 28° C for most of the year. Higher temperatures during the summer months caused inhibition of growth in many species, altered the color in the reverse of the mycelial mats, and affected the production of diffusing pigment in the medium in some species. Most of the species grew in the refrigerator at 10° C, but the rates of growth were slower. All the cultures were grown in the light and dark and the growth rates were compared.

Most of the chemicals recommended by Singer (1951, pp. 86–92) for reactions in the fruit bodies were tested on the cultures. The chemicals used with this group of species include: an alcoholic preparation of gum guaiac, 0.5 g of gum guaiac in 30 cc of 95% alcohol; 2% phenol + a few drops of aniline; 10% NH_4OH; pure aniline; 10% KOH; and 40% Formol. For the chemical tests young, actively growing cultures, 2–3 weeks old, were used. A drop of the chemical was placed on the mycelial mat and the reactions recorded as instant or occurring up to 4–6 hours later, although observations were made up to 24 hours after.

All cultures were tested for longevity after being kept for 1 year under mineral oil in the 10° C refrigerator. For this test every piece of the mat was subcultured, and although individual pieces often did not grow, growth from some part of the mat could be obtained in all species tested.

For cytological work the fungus was grown in a van Tieghem cell which

was fixed on a slide, filled with the medium, and kept in a moist petri dish. After 2–3 days and when growth was well started, the cell containing the growing inoculum was covered with a sterile 24×40 mm cover slip and in about 8–10 days, when sufficient hyphae had grown on to the cover slip, this was removed and fixed in Carnoy's fixative. The cover slips with the hyphae were stained half an hour after fixation using aqueous basic fuchsin, according to procedure I as described by DeLameter (1948). In this stain the nuclei stained bright red, the cytoplasm pale yellow, and the septa remained colorless.

In the identification of the fruit bodies Singer's (1945) taxonomic treatment of the Boletaceae was largely followed.

The color terms were obtained from Ridgway's Color Standards and Nomenclature (1912).

Taxonomic Criteria

It is thought advisable that characteristics of taxonomic significance for the whole group be discussed before proceeding to the descriptions of the individual species. It is obvious that morphological features of fruit bodies, used by the taxonomists for the delimitation of genera and species, are not present in the cultures. Instead other characteristics must be studied and compared.

Rates of Growth

Measured by the diameter of the colony in month-old cultures. The rate of growth is not claimed to be a significant taxonomic characteristic, but it is of some value at the generic level. Very few species of Boletaceae grow to cover a petri dish. The four species of *Boletinus* studied and a few species of *Suillus* reach a maximum diameter of 8 cm. The diameter attained by the majority of *Suillus* species is 6 cm and a few species do not exceed 2–3 cm. In the genus *Xerocomus*, the species studied achieve a diameter of 6 cm when grown in the dark, compared with only 4 cm when grown in the light. The *Leccinum* species reach 2–3 cm, and colonies of *Boletus* species attain similar diameters with the exception of *Boletus griseus* which grows up to 9 cm.

Color of the Mycelial Mats

Within each of the genera *Suillus*, *Boletus*, *Leccinum*, *Tylopilus*, and *Xerocomus* and to a lesser extent in the genus *Boletinus*, two groups can be distinguished on the basis of the color of the mycelial mat. In one group the mats are white or light-colored and there is no pigment in the medium, and in the other group the mats are colored and often a yellow-brown pigment diffuses into the medium.

Types of Mats

In the genera *Boletinus* and *Suillus* the mats are smooth and homogeneous with no evidence of production of fruit body initials. In the genera *Boletus*, *Leccinum*, *Xerocomus*, and *Pulveroboletus* the mats, in many species, are uneven with various outgrowths, and fruit body initials are frequently produced, although in some isolates the initials cease to appear after a few transfers. In other isolates the initials are abundant, the stipe and the pileus

become well differentiated, reaching a height of 8 cm and appearing after 2 years of subculturing. Up to the present however, only one species has developed pores, basidia, and basidiospores in culture.

In microscopic characteristics most of the species of Boletaceae show a uniformity which is annoying for the taxonomist. The differences in hyphal characteristics such as coloring, septation, and branching are more in the nature of degree than of kind. The types of hyphae are very similar to the ones found in the fruit bodies. Study of the hyphal types is best carried out using a microscope with phase contrast.

Types of Hyphae

1. The majority of the hyphae are of the ordinary type, cylindrical, hyaline, with homogeneous or granular contents, staining in KOH–phloxine, or hyaline and empty and not staining in phloxine. The staining type undergo a number of modifications and it is the degree of such modifications that varies from species to species. In the species studied the following types of modifications were observed:

(*a*) *Hyphae containing colored fluid.*—Whether these hyphae are similar to the sap hyphae found by Lohwag and Peringer (1937) in the fruit bodies of the Boletaceae is not certain. In culture such hyphae usually become wider than the ordinary (Fig. 7), their longitudinal walls remain thin, and their contents are colored pale yellow, olive yellow, or melleous. In mounts the cell wall often expands and in 10% KOH frequently breaks, the contents pouring out into the mount (Fig. 8). When a mat is touched slightly with a needle it often becomes flooded with a colored fluid similar to the one present in these hyphae. Probably the drops of exudate on the surface of some mats and the melleous drops on the surface of the sclerotia are exudations from such hyphae, and possibly the diffusing color in the medium is from the same source. The fact that the hyphae containing fluid are modified ordinary hyphae can be seen from the occurrence of normal cells and cells containing fluid on the same filament side by side, and from the occurrence of clamps on these hyphae, in those species which normally produce clamps (Fig. 9).

(*b*) *Hyphae with papillated appearance.*—These have been found and described in species of *Suillus* and *Xerocomus*. Such hyphae occur in various degrees in almost all species studied. In this case the ordinary hyphae are modified by depositions of amorphous yellow to brown material on the hyphal walls. This material does not stain in Congo red and becomes easily detached from the cell wall, floating off into the mounting fluid. The deposits are either small and rather regular in shape (Fig. 10), seen only at the edges of the hyphae, or they are large, irregular, and cover the whole surface (Fig. 11). In some species they are more firmly attached to the walls and give an ornamented appearance to the hyphae. It is assumed that this material contributes largely to the color of the mats and their reverse. In some isolates where papillated hyphae are less numerous the mats and their reverse are lighter colored.

(*c*) *Vesicular hyphae.*—These are abundant in some species and absent in others. In some species they occur in both the aerial and submerged mycelia, in others only in the submerged. The swellings are thin-walled, terminal, or

intercalary, small or very large, with the contents mostly highly granular and staining brightly in KOH–phloxine. They often become detached and float free in the mount (Figs. 12, 13).

2. *Undulate hyphae* are present in limited numbers in several species, occurring in the mycelia growing near or below the surface of the agar. They are more abundant and become almost helicoid in the surface growth in *Boletinus pictus* (Figs. 14, 15).

3. *Dendritic hyphae* are present in many species in the submerged mycelia (Figs. 16, 17).

Mycelial Strands

There are very few species of Boletaceae that do not produce strands in culture, but again the degree of strand formation varies between species. In most species the strands are formed by the parallel orientation of narrow hyphae on either side of a broad hypha (Fig. 18). In some species the strands are formed by intricately interwoven hyphae (Fig. 19). The strands may be hyaline and granular, staining in KOH–phloxine or modified to yellow or brown and papillated according to the modifications of the ordinary hyphae from which they are formed.

Hyphal Fusions

These are not frequent in most species studied. It has been observed that loss of clamps in some isolates was accompanied by an increase of hyphal fusions.

Sclerotia and Chlamydospores

So far sclerotia have been produced in one, and chlamydospores in one other, of the species studied.

Hyphal Characteristics

1. *Septation.*—In some species the septa are closer than in others, but the variation between filaments of the same species is too great to allow this characteristic to be of any taxonomic significance. The septa are simple or with clamps and with or without branching.

2. *Branching.*—There are at least two basic types of branching in the Boletaceae. In the first, just one branch is produced below the septum (Fig. 24), and in the second, two branches are formed in the same position (Fig. 25). The second type was called "paarige" by Melin and the term was retained by later authors. The pair of branches are formed at varying angles to the main hypha, are short or long, sometimes as long as the main hypha, equal or more often unequal, indicating that usually one is formed before the other. Frequency of paarige varies between species, being absent in some species, and this character is considered to be of some taxonomic significance.

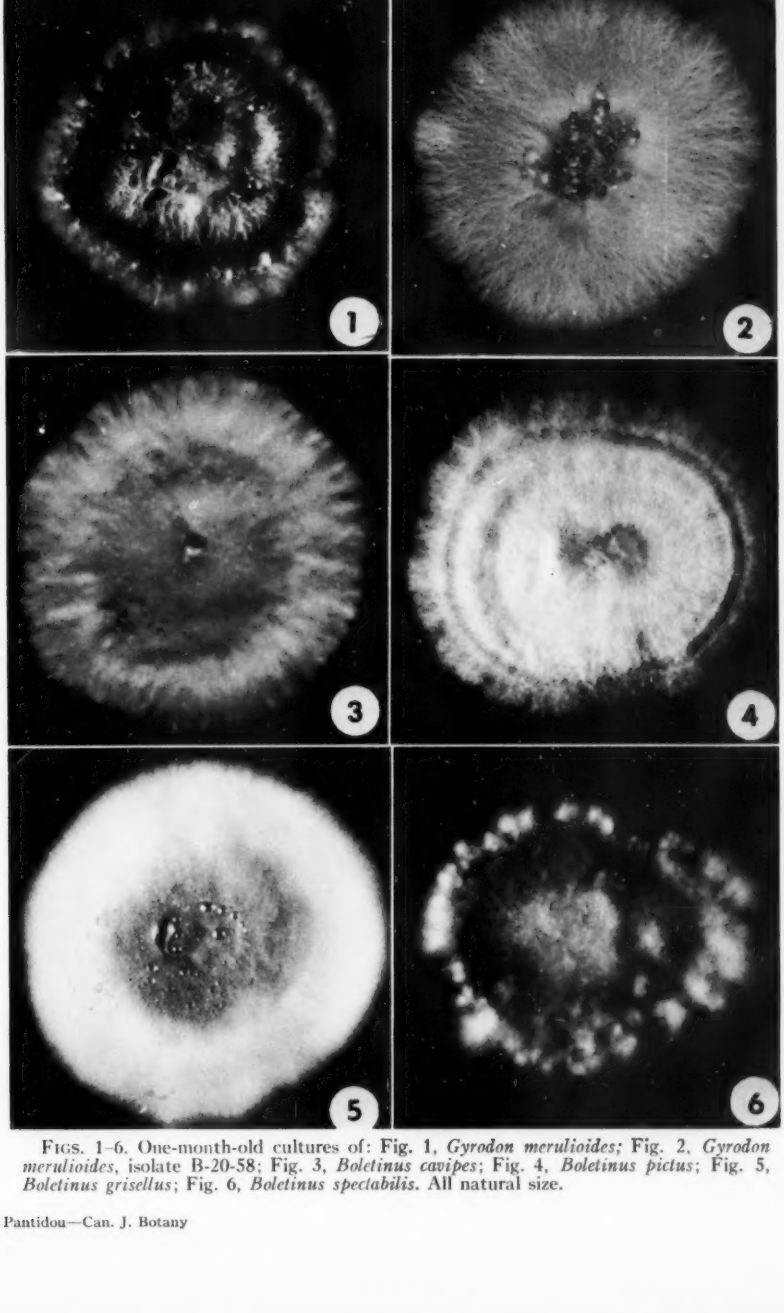
3. *Clamps.*—In all the species of *Boletinus* studied the mycelia have clamps, although there is a variation between species in the frequency, number, and position of the clamps. Singer (1951, p. 650) did not observe clamps in *Suillus*, and suggested that the presence of clamps indicated a *Boletinus*, whereas absence of clamps could mean either a *Boletinus* or *Suillus*. The cultural studies have shown, however, that all species of *Boletinus* examined have

abundant clamps, but that in *Suillus* some species have also abundant clamps, others relatively few (although constanly present), and still others appear to lack them entirely. Therefore the presence or absence of clamps cannot be used as a diagnostic character at the generic level. In the genera *Boletus*, *Leccinum*, *Xerocomus*, *Tylopilus*, and *Pulveroboletus*, clamps have never been seen. In some species clamps are found only in the aerial mycelia, in others they are frequent both in the aerial and the submerged hyphae. The occurrence of the clamps on the hyphae is not constant, since in a species bearing clamps, some hyphae have no clamps, others have clamps at every septum, and in still others clamps are present at some septa and lacking at others. In some species clamps are formed in the branches as well as in the main hyphae, in others clamps are seen only in the main hyphae. The position of the clamps in relation to the branching shows an interesting variation: some types are common in all species, while others are seen only in one or two species, and not in the others. Types noted in the five species studied include: (*a*) a clamp formed on one side of the hypha and a branch on the other (Fig. 26); (*b*) a branch formed from the loop of the clamp with the side septum present or absent (Figs. 27, 28); (*c*) a clamp formed on one side of the hypha, a branch developed normally on the other side, and this produced a clamp soon after its formation (Fig. 29); (*d*) a branch formed from the loop of the clamp produced another clamp soon after its inception (Figs. 29, 30) (the last type is seen only in *Gyrodon merulioides* and *Boletinus cavipes*); (*e*) paarige with one branch developed from the loop of the clamp, the other not, with side septa present or absent (Figs. 31, 32) (this type is very common in all the five species studied); (*f*) paarige with three clamps, one formed on the main hypha, the other two on the branches soon after their inception (this type is seen only in *Gyrodon merulioides* and *Boletinus cavipes* (Fig. 33)); (*g*) three branches formed in the same position, clamp in the main hypha present or absent (this type, so far, is seen only in *Boletinus spectabilis* (Figs. 34, 35)).

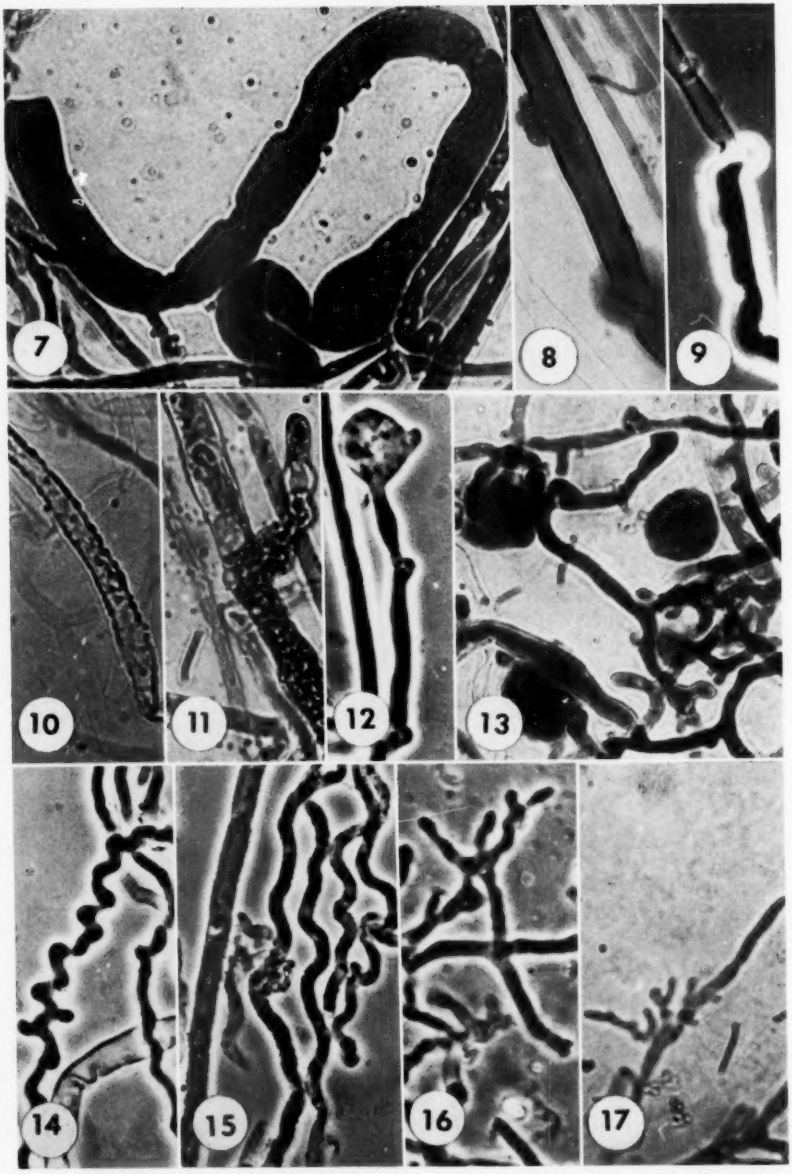
Chemical Tests

The test for the presence of an oxidase by means of an alcoholic preparation of gum guaiac (Nobles 1958) has shown some interesting results. In the section *Versipelles* of the genus *Leccinum*, cultures of five species tested showed a strong spot reaction, the color ranging from marine blue to Capri blue or blackish green-blue. It is interesting to note that the same test when applied to the context of the pileus of a fruit body proved negative. This is the only section in which an almost instant reaction has been observed in every species, but individual species in other groups may show it as well. For example, *Suillus americanus* shows a strong blueing reaction and in this case the blueing is strong in the context of the fruit body. In another group of species, from different genera, there is a delayed reaction, and in still others the test is always negative.

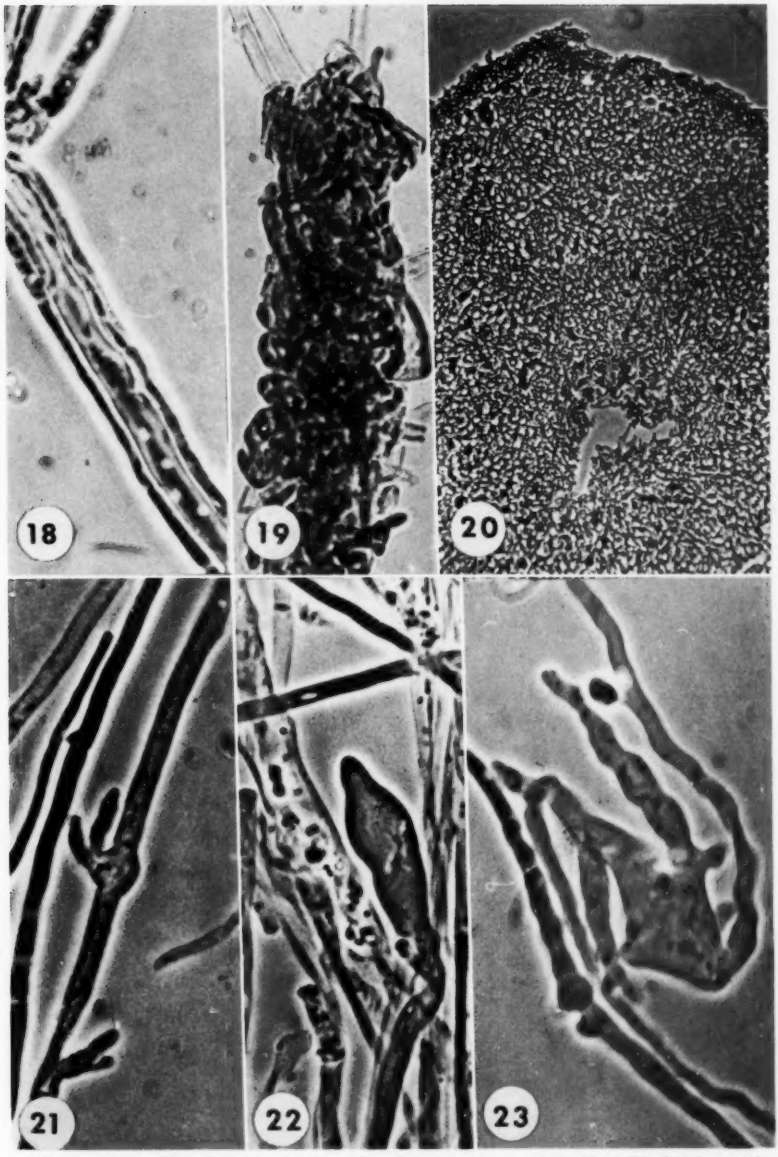
Many species of Boletaceae show a positive reaction with chemicals with a hydroxyl radical, of which the most active proved to be with NH_4OH. Reactions with this and other chemicals will be presented in the descriptions of the individual species. Color reactions between the fungous mats and



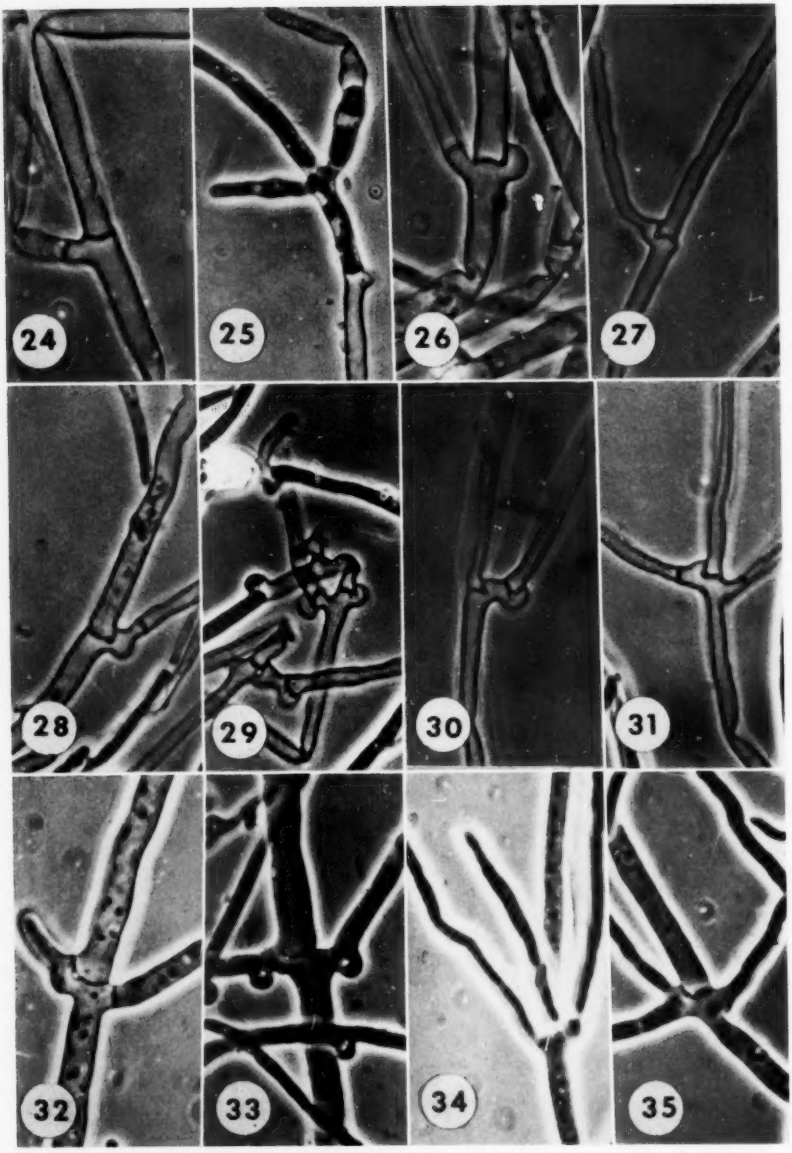
FIGS. 1–6. One-month-old cultures of: Fig. 1, *Gyrodon merulioides;* Fig. 2, *Gyrodon merulioides*, isolate B-20-58; Fig. 3, *Boletinus cavipes*; Fig. 4, *Boletinus pictus*; Fig. 5, *Boletinus grisellus*; Fig. 6, *Boletinus spectabilis.* All natural size.



FIGS. 7, 8, 9. Hyphae containing fluid, 9 under phase contrast. FIGS. 10, 11. Papillated hyphae. FIGS. 12, 13. Vesicular hyphae, 12 under phase contrast. FIGS. 14, 15. Undulate to helicoid hyphae of *Boletinus pictus*, both under phase contrast. FIGS. 16, 17. Dendritic hyphae, both under phase contrast. All ×900.



FIGS. 18, 19. Mycelial strands, 18 under phase contrast, ×900. FIG. 20. Section with freezing microtome of a sclerotium of *Gyrodon merulioides*, ×370. FIGS. 21, 22, 23. Branching, cystidium, and type of swollen hypha in *Boletinus grisellus*; all under phase contrast; all ×900.



FIGS. 24–33. Configurations of branches and clamps (see text, pp. 1153–54); all under phase contrast; all ×900. FIGS. 34, 35. Unusual branching in *Boletinus spectabilis*; both under phase contrast; both ×900.

various chemicals seem promising and offer scope for biochemical investigation.

Longevity

With very few exceptions the cultures of Boletaceae never use all the available food in the medium; their growth rate falls off at an early stage and they die. Certain changes have been observed accompanying the inhibition of growth, such as color change in the mats and their reverse, collapse of the mycelium in the center of the mat, and irregular growth at the margin, which may become hyaline or dendritic or produce sectors. These changes are pronounced in some species but almost absent in others.

Descriptions of Cultures

Gyrodon merulioides (Schw.) Sing., Rev. mycol. **3**, 172 (1938)

Macroscopic Characteristics

Mycelial mat (Fig. 1) with aerial and submerged growth present, the former light ochraceous buff or warm buff, the latter tawny olive or buffy brown. Margin regular or irregular in outline, light ochraceous buff. Reverse snuff brown to bister to sepia to ochraceous tawny. A tawny olive pigment diffusing into the medium in some isolates. The maximum diameter of growth in month-old cultures varying from 3 to 7 cm in the different isolates, the same in light or dark. In month-old cultures marginal growth becoming transparent, irregular, and often dendritic. All isolates with or without mineral oil remaining alive in the refrigerator after 1 year.

Sclerotia present in all isolates, more abundant in cultures grown in dark than in light and more abundant in the range of 0.2–1.5% of malt extract in the medium. Their formation checked at 0.1% and again at 2% of malt extract. Sclerotia beginning to appear about 2 weeks after inoculation, often accumulating at the very center of the culture and becoming completely covered by the aerial mycelium. The individual sclerotia superficial, small, 0.1–0.5 cm in diameter, dark brown, almost black, often covered with tawny olive strands of mycelium and drops of a melleous exudate; globose to cylindrical in shape, single or coalescing in groups of 2–10, rather soft in consistency. Rind of sclerotium consisting of 3–4 layers of small isodiametric cells heavily impregnated with brown material; tissue of the medulla pseudoparenchymatous, consisting of large hyaline cells, empty or with granular contents, some of the last staining deeply in KOH–phloxine; center of the sclerotium hollow, lined with cylindrical hyphae heavily impregnated with brown material (Fig. 20).

Microscopic Characteristics

In the marginal growth, hyphae hyaline with homogeneous or granular contents and staining brightly in phloxine, very few modified hyphae present. In the center of the mat, the majority of the hyphae 3–7 μ in diameter, hyaline, with the contents mostly granular, some appearing empty and not staining in phloxine, few reaching a diameter of 14 μ and containing a bright yellow to melleous fluid. Few papillated hyphae present. In some areas of the mat, hyphae forming intercalary or terminal vesicles (Figs. 12, 13), usually staining

brighter than the ordinary hyphae, and a few becoming filled with colored fluid. In the submerged mycelia, dendritic hyphae numerous, predominantly hyaline, but a large proportion of vesicular and papillated hyphae present.

Septa close, mostly simple in the marginal hyphae, becoming regularly clamped in the older, in both the aerial and the submerged mycelia. Branching not frequent in the marginal hyphae, abundant in the remainder, simple or paarige. Clamps present in all the arrangements shown in Figs. 26–33, formed in the main hyphae as well as in the branches. Paarige with three clamps more frequent than in *Boletinus cavipes*.

Hyphal fusions rare. Strands abundant, some up to 115 μ wide, mostly formed by intricately interwoven hyphae (Fig. 19), the majority hyaline and staining brightly in KOH–phloxine when young, later becoming yellow or brown, depending on the degree of modification of the ordinary hyphae.

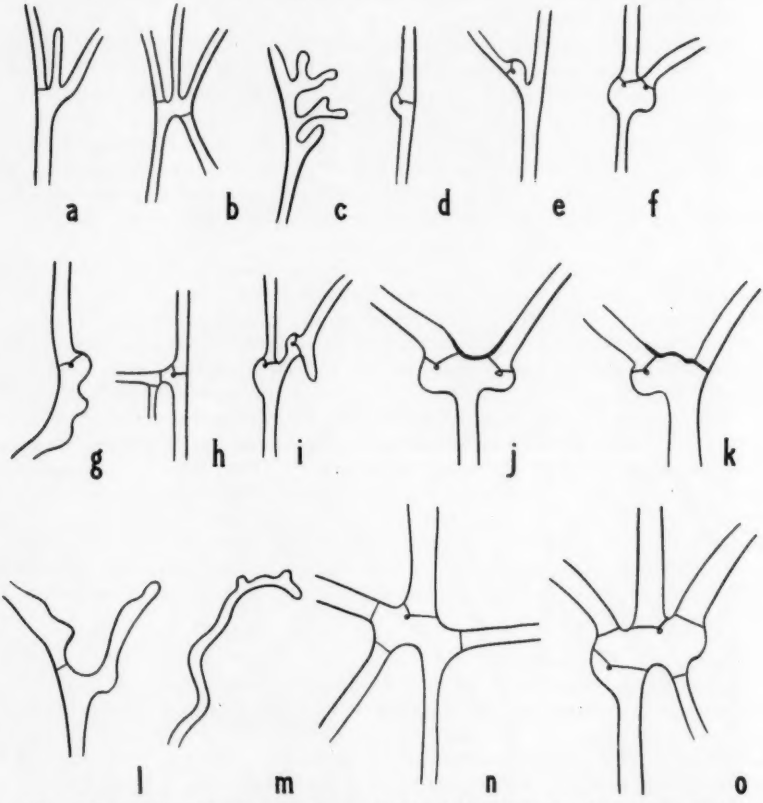


FIG. 36. *a–c*. Branching in *Gyrodon merulioides*, isolate B-20-58. *d–f*. Clamps in fruit body of *Gyrodon merulioides* isolate B-20-58. *g–k*. Unusual branching in *Boletinus cavipes*. *l–o*. Unusual branching in *Boletinus spectabilis*. All ×1000.

Chemical Reactions

Gum guaiac, negative or a very slight delayed reaction; *phenol + aniline*, a spot reaction of neutral red; *NH_4OH*, the whole mat turning fawn color or light vinaceous fawn; *pure aniline*, negative; *KOH*, negative; *Formol*, negative.

In one isolate, B-20-58 (Fig. 2), the culture showed certain differences and this isolate is believed to be a haploid. The mycelial mat was much lighter in color, light buff to cream color, and the growth was appressed and partly submerged with almost no aerial mycelium. The reverse was also lighter, warm buff to ochraceous salmon. The sclerotia were exposed from the beginning since aerial hyphae were lacking. Diffusing pigment was never produced. In mounts the hyphae were narrow, 2–3 μ, almost all hyaline, very few being modified to the fluid-containing type. Papillated hyphae were almost absent. The surface hyphae were rarely branched and the ones close to the agar showed the irregular branching common in the submerged mycelia (Fig. 36, *a*, *b*, *c*). Clamps have never been seen, even after long searching. Hyphal fusions increased in frequency. Cytological work has shown that the hyphal cells were uninucleate, whereas those of the other isolates were binucleate. The fruit body of the isolate B-20-58 was at first thought to be haploid but examination of hyphae from the fruit body showed mycelia with clamps (Fig. 36, *d*, *e*, *f*). Since the culture was without clamps from the beginning it is possible that the reversion to the clampless and uninucleate condition occurred immediately the tissue was cultured.

A second case of reversion to the clampless condition has occurred more recently in another isolate from Indiana, B-22-58. After 2 years of subculturing, this isolate, which initially showed abundant clamps, lost its clamps, became lighter in color, lost its aerial growth, increased the formation of hyphal fusions, and became similar in appearance to B-20-58 above.

Cultures Studied

B-19-58 (fruit body DAOM 61843, collected Ottawa, Ontario); B-20-58 (fruit body DAOM 61845, collected Kingsmere, Quebec); B-21-58 (fruit body DAOM 61846, collected Ottawa, Ontario); B-22-58 (fruit body DAOM 61847, collected Bloomington, Indiana, U.S.A.); B-226-60 (fruit body DAOM 71902, collected Conway, Massachusetts, U.S.A.).

Boletinus cavipes (Opat.) Kalchbr., Botan. Z. **25**, 181 (1867)

Macroscopic Characteristics

Mycelial mat (Fig. 3) snow white in the beginning, developing a straw yellow to warm buff coloring in 2–3 weeks, deepening to honey yellow with time, this taking place sooner in the light than in the dark. Cultures grown in the dark remaining white for a longer period of time and when removed to light the yellow color developing in a few hours. In freshly isolated cultures a red color appearing when mat touched with a needle. Aerial mycelium high, reaching the lid of the plate, cottony, little or no submerged growth present, and the mat separating readily from the surface of the agar. Margin raised, regular in outline, growth often in tufts. Reverse light ochraceous salmon in the center, the remainder light buff. Maximum diameter of growth 7–8 cm in one month, slightly more in the dark. Odor aromatic. No diffusing pigment in the medium. Often a yellow or orange exudate present on the top

of the mat at the point touching the lid, possibly contact with the glass causing the hyphae containing fluid to break. In month-old cultures marginal growth becoming submerged and transparent, often sectoring, the aerial part collapsing and becoming a mixture of dirty whitish and buffy brown, the reverse olive brown. Cultures under mineral oil remaining alive for 1 year in the refrigerator but cultures without oil dying in 1–2 months.

Microscopic Characteristics

Hyphae with homogeneous contents, staining in KOH–phloxine, separating easily from each other and rarely forming strands or mycelial sheets. Marginal hyphae predominantly narrow, 2–3 μ, in the rest of the mat ranging from 2 to 7 μ, with a few up to 10 μ wide. Modifications of the ordinary hyphae very limited, the modified hyphae containing fluid in larger proportion than the others, pale yellow, few bright yellow or orange in the honey-colored part of the mat. Short, wide, very closely septate hyphae often present with walls and cross septa up to 1 μ thick, staining in Congo red, and containing a pinkish or reddish coloring in the cytoplasm in the freshly isolated cultures, causing a red coloring on the mat when touched for transfer, in the older cultures becoming hyaline and rare. In the mat next to the agar some hyphae with uneven walls (Fig. 36, *g*). Strands not frequent, more abundant in the older cultures and in the older portion of the mat.

Septa distant, formed well behind the growing tip. Most hyphae with repeated branching along their line of growth. Clamps present in all the arrangements shown in Figs. 31–35. Paarige frequent, but presence of three clamps rare. Secondary branches seen in a few instances (Fig. 36, *h*, *i*). Main hyphae occasionally dividing dichotomously, developing one or two clamps at the point of division, the last appearing thickened (Fig. 36, *j*, *k*).

Chemical Reactions

Gum guaiac, negative; *phenol + aniline*, a spot reaction of brick red in half an hour, the coloring spreading with time; *NH_4OH*, negative; *KOH*, negative; *Formol*, negative.

This is another species in which a change occurred. One isolate lost its abundant clamps after a year of subculturing. Cytological investigation showed that the hyphal cells remained binucleate. Morphologically the culture lost its fluffy mycelium, became appressed, slower in growth, and died.

Cultures Studied

B-1-57 (fruit body DAOM 57609, collected Ramsayville, Ontario); B-28-58 (fruit body DAOM 61851, collected Lake Opinicon, Ontario).

Boletinus pictus (Peck) Peck, Bull. N.Y. State Museum, **8**, 77 (1889)

Macroscopic Characteristics

Mat (Fig. 4) smooth, furry to velvety, appressed. Margin regular in outline, appressed. Maximum diameter of growth 5–6 cm. Color of the mat undergoing a number of changes. When first cultured from the tissue, one-week-old cultures whitish with pallid mouse gray to gull gray tones, later changing to pale pinkish cinnamon or pale yellow-orange to capucine buff or pale ochra-

ceous buff. After several transfers the mouse gray tones disappearing in the young cultures, and the colors mostly in the pale cinnamon or ochraceous buff range. Similar changes apparent in the reverse, in newly established cultures the reverse being dull purplish black or deep mouse gray, after several transfers fading to cinnamon buff or olive brown or olive buff in the center, buffy brown in the margin, sometimes more or less zoned. Mat with submerged mycelia and not separating from the agar. Diffusing pigment never produced. In month-old cultures growth ceasing completely and margin appearing dark because of the colored reverse, or growth continuing, hyaline and uneven. Center of the mat collapsing and becoming vinaceous buff to tilleul buff, and the reverse dark vinaceous drab. Diameter of growth same in light and dark. Cultures surviving 1 year storage under mineral oil in the refrigerator, not living more than 3–4 months without it.

Microscopic Characteristics

Hyphae 2.5–5 μ wide, the majority with straight walls but a large number of very narrow, undulate to almost helicoid hyphae always present (Figs. 14, 15). Even at the margin, hyphae close-packed forming a smooth layer, and individual hyphae partly losing their identity. In the center of the mat, fusion of the individual hyphae to form a smooth mycelial sheet more pronounced, but the helicoid hyphae retaining their identity. Some papillated hyphae present, more numerous in the submerged mycelia. Strands present, formed by parallel arrangement of the hyphae, mostly hyaline. Hyphae with fluid contents rare, and no vesicular hyphae seen.

Septa rather close, simple or clamped. Branching not as frequent as in the other species, and formation of one branch more frequent than paarige. Clamps always present but not abundant, more frequent in the paarige; always in the main hypha, not seen in the branches.

Chemical Reactions

Chemically, the most active species studied. ***Gum guaiac***, no blueing in freshly isolated cultures, but the spot instantly turning Pompeian red or salmon color or fawn color; after 1–2 years of subculturing the same isolates developing an instant spot color of pale vinaceous, this changing to blue in 15–20 minutes, probably indicating an increased production of oxidase as a result of growth on this medium, the initial red coloring possibly being due to a precursor of the oxidase; ***phenol + aniline***, a spot reaction of vinaceous or salmon color; ***NH_4OH***, instantly coloring the whole mat pale vinaceous or Laelia pink; ***pure aniline***, coloring the spot and gradually the whole mat rose or Laelia pink or russet vinaceous; ***KOH***, coloring the mat pale vinaceous fawn in 4–5 hours; ***Formol***, coloring the mat pale vinaceous fawn in 4–5 hours.

Cultures Studied

B-23-58 (fruit body DAOM 61848, collected South March, Ontario); B-24-58 (fruit body DAOM 61849, collected Ramsayville, Ontario); B-25-58 (fruit body DAOM 61850, collected Lake Opinicon, Ontario); B-108-59 (fruit body DAOM 65192, collected Baie St. Paul, Quebec); B-141-60 (fruit body DAOM 74128, collected Bell's Corners, Ontario).

Boletinus spectabilis (Peck) Murrill, N. Am. Flora, **9**, 160 (1910)

Macroscopic Characteristics

The most variable of the Boletinus species studied. When first isolated, slow and irregular in growth, after several transfers becoming faster and more regular in outline, the particular medium employed apparently favoring this species. Mat with thin aerial mycelium, whitish or pale cinnamon pink, and submerged growth present, the latter becoming colored cinnamon buff to cinnamon in parts off the center of the mat, margin even or uneven, often with a white growth (Fig. 6). Thick mycelial strands, bright yellow or cinnamon in color, often visible on the mat. Reverse a mixture of olive-ocher, cream buff, and tawny olive. In the early stages of growth olive-ocher pigment diffusing into the medium but not evident as the mat increases in diameter. In older cultures aerial mycelium pale vinaceous fawn, the center buffy brown, and the margin cinnamon. Reverse Verona brown to Sayal brown in the center, fading to cinnamon in the margin. Maximum diameter of growth 5–6 cm in a month. Cultures surviving 1 year storage under mineral oil in the refrigerator but not living more than 2–3 months without it.

Microscopic Characteristics

Hyphae hyaline, variable in diameter, 3–7 μ, some up to 10 μ, predominantly hyaline and granulated, staining in KOH–phloxine, or hyaline and empty and not staining. Modified hyphae of the papillated type, abundant; of the fluid-containing type, moderately abundant. The papillated type more abundant in the submerged mycelia, with walls more or less covered with dark yellow to cinnamon deposits (Fig. 11), becoming easily detached, and floating into the mounting fluid. Strands abundant, formed by parallel orientation of hyphae, hyaline and staining in phloxine in young cultures, becoming dark yellow or cinnamon with heavy deposits in the older cultures. Hyphae with uneven walls (Fig. 36, *l*, *m*), and dendritic hyphae abundant in the submerged mycelia. The large number of papillated hyphae, the most abundant waste material, and darker colors of the mat and the reverse, distinguish this species from any other of the *Boletinus* species studied.

Septa close, simple, or clamped. Paarige-branching frequent with or without a clamp at the point of branching. Clamps seen only on the main hypha, not in the branches. In this species often three instead of two branches present (Figs. 34, 35), the third branch often growing backwards (Fig. 36, *n*). In another unusual type the main hypha developed a clamp, and soon after it branched to a paarige with one branch grown from the loop of the clamp and developing a secondary branch backwards (Fig. 36, *o*). Clamps not seen in the submerged hyphae.

Chemical Reactions

Gum guaiac, negative; *phenol + aniline*, negative; *NH_4OH*, coloring instantly the whole mat pale purplish vinaceous or purplish vinaceous; *pure aniline*, coloring the spot light russet vinaceous in 15 minutes, color spreading with time; *KOH*, coloring the spot purplish vinaceous in 5–10 minutes; *Formol*, coloring the mat pale vinaceous in 5 hours.

Cultures Studied

B-26-58 (fruit body DAOM 61852, collected Lake Opinicon, Ontario);

B-285-60 (fruit body DAOM 74123, collected Paul Smith, N.Y., U.S.A.).

Boletinus grisellus Peck, Mem. N.Y. State Museum, **3**, 169 (1900)

Macroscopic Characteristics

The young cultures, 2 weeks old, white and slightly fluffy, later the center changing to tilleul buff, often covered with hyaline drops, the rest remaining white, losing its fluffiness, and forming a very thin, smooth layer on the agar. The margin regular in outline and appressed (Fig. 5). Maximum diameter of growth 6–7 cm in a month. Reverse pale pinkish buff all over. In older cultures marginal growth becoming hyaline and transparent, and the reverse changing to buffy brown. No diffusing pigment. Under mineral oil, cultures surviving 1 year storage in the refrigerator but cultures not under oil dying after 2–3 months.

Microscopic Characteristics

Hyphae 3–4 μ wide, forming a compact layer, the majority of the ordinary type, granular and staining in *KOH*–phloxine or empty and not staining. Hyphae with fluid rare, and their contents pale yellow or almost colorless. Papillated hyphae with pale yellow depositions more numerous. Strands not seen.

Septa distant, some simple but a large proportion clamped. Paarige-branching not as frequent as in the other species of *Boletinus*, usually one clamp present in the main hypha, but no clamps seen in the branches. Some hyphae in this species producing peculiar branches (Fig. 21) or losing their cylindrical shape and becoming irregularly swollen (Fig. 23). Cystidiumlike bodies also present (Fig. 22), staining brightly in phloxine.

Chemical Reactions

Gum guaiac, negative; *phenol* + *aniline*, negative; *NH_4OH*, instantly coloring the whole mat or part of it pale purplish vinaceous; *pure aniline*, negative; *KOH*, negative; *Formol*, negative.

In the morphology of the fruit body this species is very similar to *Suillus aeruginascens* and two species can be easily confused in the field. In culture the two species have different coloring in the reverse of the mats, and in this species clamps are always present, whereas in S. *aeruginascens* clamps have never been seen, and the modifications of the hyphae are different.

Culture Studied

B-68-58 (fruit body DAOM 61872, collected Lake Opinicon, Ontario).

Summary

The main characteristics distinguishing the species described above can be summarized as follows:

Gyrodon merulioides.—Mat colored tawny olive and ochraceous buff. Diffusing pigment often present. Sclerotia present in all isolates. Strands abundant. Clamps abundant in both the aerial and submerged mycelia, formed on the main hyphae as well as on the branches. Paarige with three clamps frequent. No reaction with the chemicals tested, except a slight reaction with NH_4OH.

Boletinus cavipes.—Mat snow white, aerial growth reaching the lid of the

plate. Modifications of the ordinary hyphae moderate. Clamps abundant in the aerial hyphae, rare in the growth near the agar, present in the main hyphae as well as in the branches. Paarige with three clamps rare. No reaction with the chemicals tested, except a delayed spot reaction with phenol + aniline.

Boletinus pictus.—Mat with mouse gray tones in the freshly isolated cultures. Modifications of the ordinary hyphae limited. Undulate to helicoid hyphae present. Clamps not abundant, formed only in the main hyphae, not in the branches. Paarige not frequent. Chemically the most active species (see description).

Boletinus spectabilis.—Mat with cinnamon colors. An olive-ocher pigment diffusing in the medium in the young cultures. Heavily papillated hyphae and waste material abundant. Strands abundant. Clamps only on the main hyphae, not formed in the branches. Paarige frequent, often three branches present instead of two. Negative to all chemicals except NH_4OH.

Boletinus grisellus.—Mat a very thin layer, white but changing to tilleul buff in the center. Modifications of the ordinary hyphae very limited. Irregularly swollen hyphae and cystidiumlike bodies present. Paarige rare. Clamps only on the main hyphae, not formed on the branches. Negative to all chemicals except NH_4OH.

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References

DeLAMETER, E. 1948. Basic Fuchsin as a nuclear stain for fungi. Mycologia, **40**, 423–429.

HOW, J. E. 1940. The mycorrhizal relations of Larch I. Study of *Boletus elegans* Schum. in pure culture. Ann. Botany, N. S. **4**, 135–150.

KHUDYAKOV, Y. P. and VOZNYAKOVSKAYA, V. M. 1951. Pure cultures of mycorrhizal fungi. Mikrobiologiya, **20**, 13–19. (In Russian.)

LOHWAG, H. and PERINGER, M. 1937. Zur Anatomie der Boletaceae. Ann. Mycol. **35**, 295–331.

MELIN, E. 1922*a*. *Boletus*-Arten als Mykorrhizapilze der Waldbäume. Ber. deut. botan. Ges. **40**, 94–97.

——— 1922*b*. Untersuchungen über die *Larix*-Mykorrhiza, I. Synthese der Mykorrhiza in Reinkultur. Svensk Botan. Tidskr. **16**, 161–196.

——— 1923*a*. Experimentelle Untersuchungen über die Birken- und Espenmykorrhizen und ihre Pilzsymbioten. Svensk Botan. Tidskr. **17**, 479–520.

——— 1923*b*. Experimentelle Untersuchungen über die Konstitution und Ökologie der Mykorrhizen von *Pinus silvestris* L. und *Picea abies* (L.) Karst. Mykol. Untersuch. und Ber. von R. Falck, **2**, 73–331.

MIKOLA, P. 1955. Metsämaan kantasienien puhdasviljely. Karstenia, **3**, 5–16.

MODESS, O. 1941. Zur Kenntnis der Mykorrhizabildner von Kiefer und Fichte. Symbolae Botan. Upsalienses, **5**, 1–147.

NOBLES, M. K. 1958. A rapid test for extracellular oxidase in cultures of wood-inhabiting Hymenomycetes. Can. J. Botany, **36**, 91–99.

RIDGWAY, R. 1912. Color standards and color nomenclature. Washington, D.C.

SINGER, R. 1945. The Boletineae of Florida with notes on extralimital species II. The Boletaceae (Gyroporoideae). Farlowia, **2**, 223–303.

——— 1951. The Agaricales. Lilloa, **22**, 86–92, 650.

SOBOTKA, A. 1954. Ciste Kultury z plodnic mykorrhitickych hub. Prace vyzkum Ust. lesn. CSR, **6**, 101–123.

——— 1955. Pestovani mykorrhiznich hub v citych kulturach. Sbornik Ceskoslov. akad. zemedel. Praha, **28**, 332–335.

CARPOPHORES OF PHLEBOPUS SULPHUREUS IN CULTURE[1]

MARIA E. PANTIDOU

Abstract

Phlebopus sulphureus (Fr.) Sing., possibly a mycorhizal fungus, produced mature carpophores in culture. This is the first report of a species of Boletaceae fruiting in culture. The developmental stages of the fruit bodies are described.

Introduction

In a first communication on cultural studies of Boletaceae (1961), it was mentioned that some species in the genera *Boletus*, *Leccinum*, *Xerocomus*, and *Pulveroboletus* produce fruit body initials in culture. In most cases the carpophore initials produce a stipe and a rudimentary pileus, in some instances however, as in one species of *Xerocomus*, the stipes are rudimentary and the pilei become well developed, colored dark brown, and the tubes are well formed but remain white and gelatinous. Basidia with sterigmata are often present but no spores have been produced. So far only in *Phlebopus sulphureus* have the carpophores developed normally with the tubes maturing and producing spores.

The isolate of *P. sulphureus* was obtained from a fruit body collected in Walker, Louisiana, during the 1960 Mycological Foray. This isolate proved very interesting in both its mycelial and its fructifying stages.

Description of the Culture

Macroscopic Characteristics

Mycelial mat with most of its growth submerged. Margin hyaline, merging into Sayal brown or tawny olive, the center snuff brown. A thin superficial growth of light grayish olive or drab often present and cream buff strands traversing the mat irregularly (Fig. 4). Reverse with a narrow hyaline margin, next Sayal brown, and the center bister. Diffusing pigment present or absent. Diameter of growth in month-old cultures 4–7 cm. Odor faint but characteristically phenolic.

Microscopic Characteristics

Hyphae hyaline, narrow, 2–3 μ, rarely branched, few covered with yellow papillations, the majority forming chlamydospores, a very small proportion remaining intact. The chlamydospores are delimited between two septa, and frequently a segment of hyaline empty hypha, with another septum, remains in between two chlamydospores (Fig. 1). They are hyaline and staining at first, later turning yellow, and finally becoming rounded with thick olive yellow walls and yellow granular contents, a large number becoming separated with the hyaline hypha remaining attached to one or both ends of the spore. Variable in size 7–12$\times$3.5–7 μ and cylindrical, oblong, or rounded in shape.

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Strands rather rare, formed in a regularly intertwined pattern (Fig. 2), their individual hyphae often forming chlamydospores.

Chemical Reactions

Gum guaiac, negative, or a very slight blueing in the margin; *phenol + aniline*, negative; *NH_4OH*, slowly changing the color of the mat to dark vinaceous; *KOH*, negative; *Formol*, negative.

Development of the Carpophore

Initially, one fruit body developed in a month-old culture in a petri dish. It grew at the very edge of the mat, on the marginal hyaline mycelium. In later subcultures the time for the initiation of the primordia was shortened, and several primordia often appeared in a single culture. Primordia have developed in cultures from 1 week to 2 months old, often 20 or more primordia being produced in a single culture, usually 2–4 of them maturing to normal fruit bodies, the others remaining undeveloped or producing abnormal carpophores.

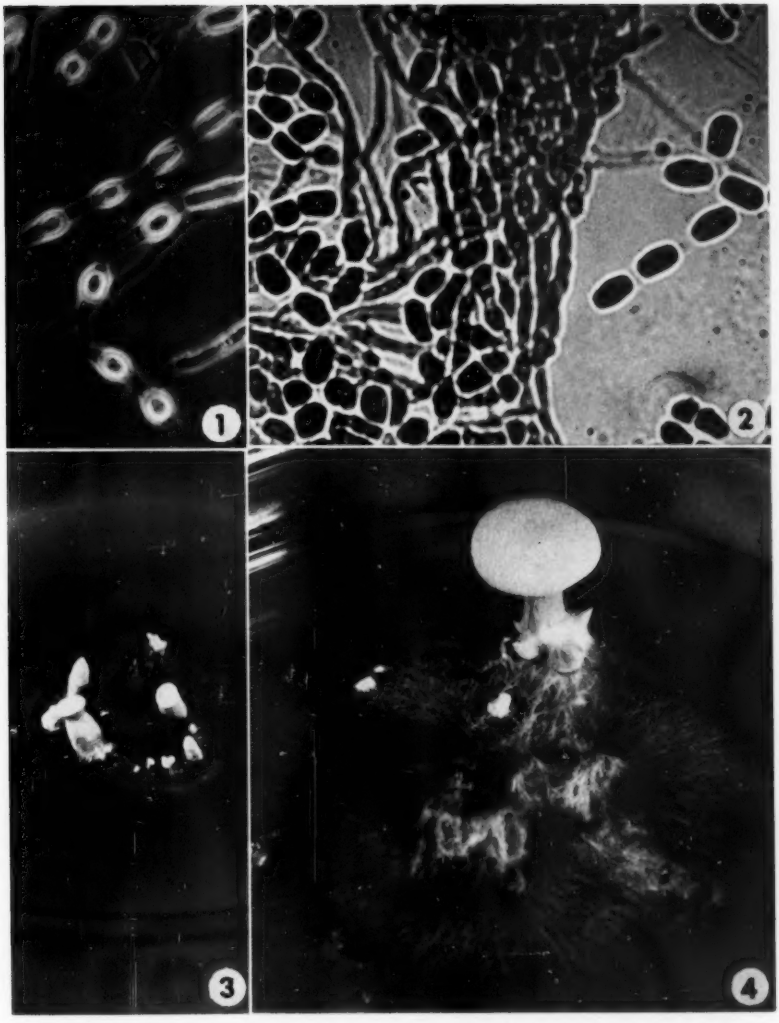
Developmental Stages

The material for study was fixed in a mixture of ethyl alcohol, propionic acid, and formalin for several days. This hardened the tissue sufficiently to permit sectioning with the freezing microtome. Sections 15–20 μ thick were obtained and they were stained with cotton blue or phloxine.

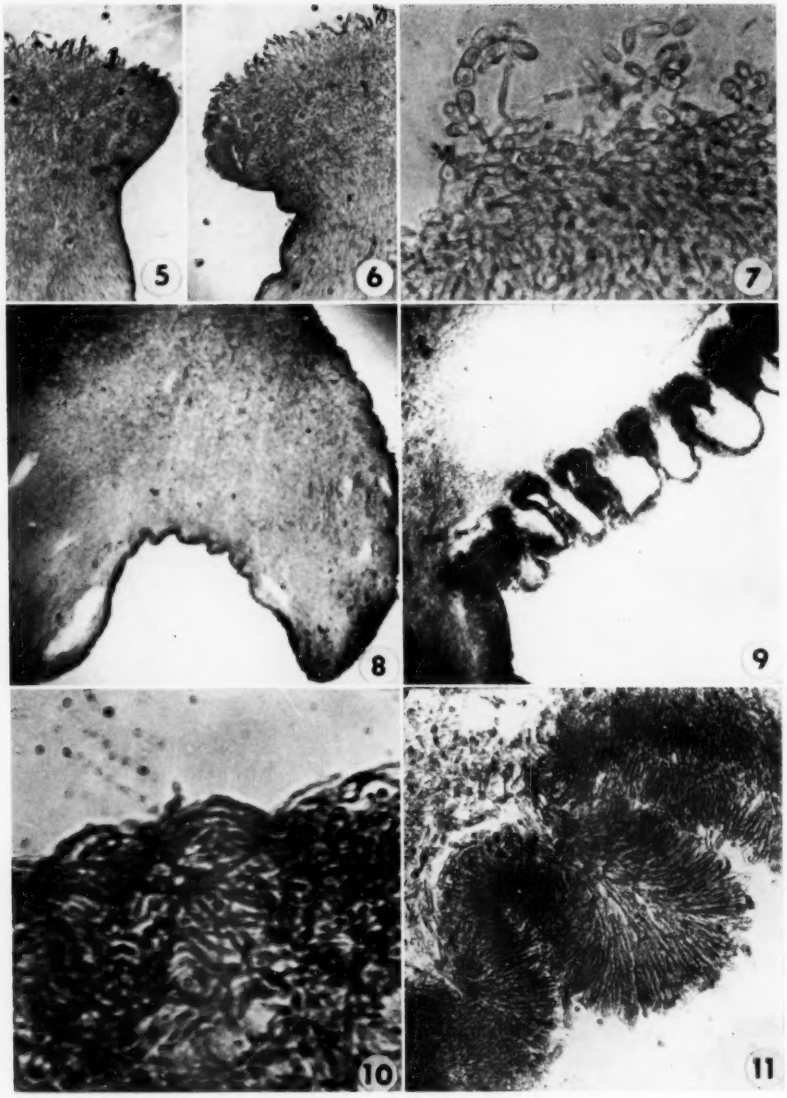
The primordium appears as a tiny dot, easily detected from its earliest initiation by its bright yellow color. Sections at this stage show an irregular agglomeration of hyphae, hyaline or bright yellow in color, presenting a sharp contrast to the hyphae of the substratum which consist of dark yellow chlamydospores.

The primordium grows to about 2–4 mm in height in 24–48 hours, and often at this stage the first differentiation is apparent. The summit of the primordium becomes more or less pointed (Fig. 3), paler yellow in color, sometimes almost white. In section this part consists of loosely arranged, hyaline hyphae which stain deeper than the rest of the hyphae in the primordium.

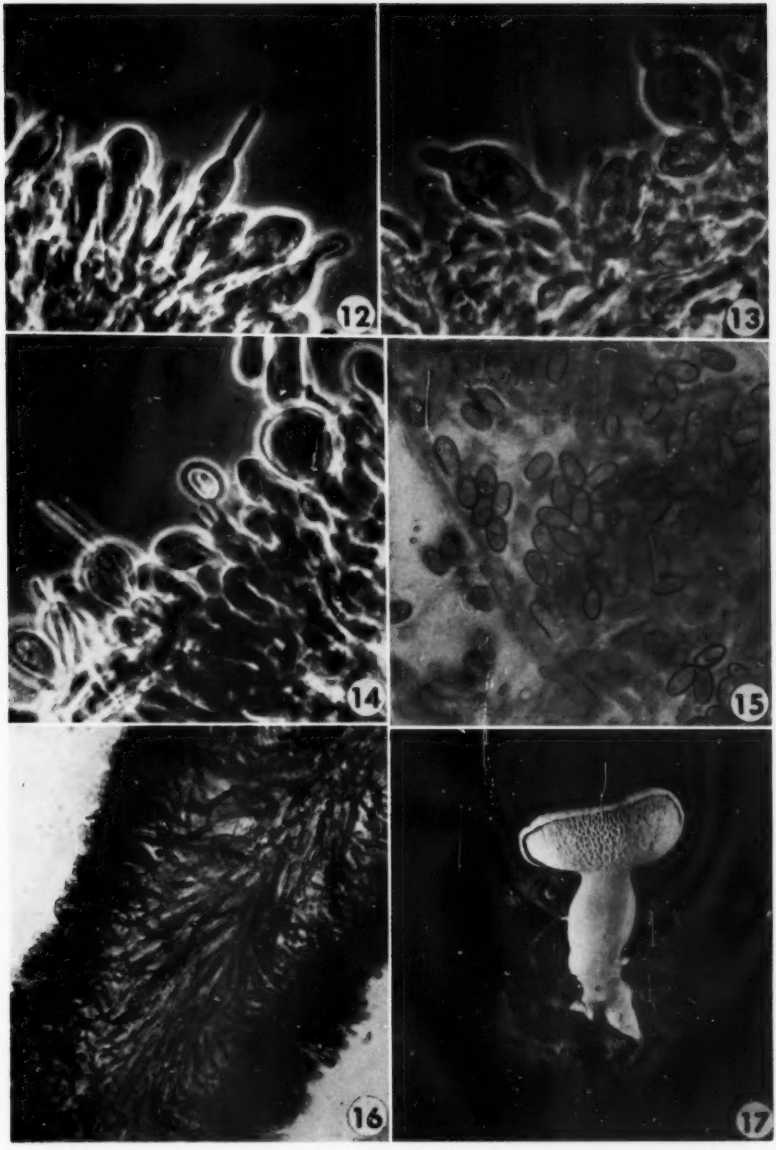
In another 24–48 hours the primordium grows to 4–8 mm in height and the differentiation into pileus and stipe is complete. The stipe appears constricted at the apex and the pileus has the regular shape of a mature carpophore (Fig. 3). At this stage the color of the pileus is lemon yellow or Pinard yellow and that of the stipe chrome yellow or buff yellow. The lower surface of the pileus is smooth, concolorous with the stipe, and there is no trace of tube formation. In section the summit of the pileus consists of loosely arranged erect hyphae, sometimes in tufts, mostly hyaline, often a number of them forming chlamydospores which are young and hyaline (Figs. 5, 7). On the lower side of the pileus, starting from an area close to the margin, the hyphae have formed a palisade layer which extends along the lower surface of the pileus and down some distance on the surface of the stipe (Fig. 5). It appears that hyphae of the context orient themselves perpendicular to the surface and become aligned side by side, forming a compact palisade, with the rounded ends of the hyphae pointing outwards. The cells of the palisade and parti-



FIGS. 1–4. *Phlebopus sulphureus*. Figs. 1, 2. Chlamydospores and mycelial strand, 1 under phase contrast, both ×900. Figs. 3, 4. Mycelial mat, primordia, and mature carpophore, all natural size.



FIGS. 5–11. *Phlebopus sulphureus*. Figs. 5, 6, 8. Stages in the development of the primordium, all ×22. Fig. 7. Cuticular hyphae of the pileus in the primordium forming chlamydospores, ×540. Fig. 9. Boletinoid type of hymenophore, ×12. Fig. 10. Cutis of the pileus in the mature carpophore, ×370. Fig. 11. Early stage in the formation of the hymenophoral trama, ×220.



FIGS. 12–17. *Phlebopus sulphureus*. Figs. 12, 13. Cystidia of the hymenium, under phase contrast, ×900. Figs. 14, 15. Basidium and basidiospores, 14 under phase contrast, both ×900. Fig. 16. Bilateral and divergent hymenophoral trama, ×222. Fig. 17. Mature carpophore, showing the gyrodonlike tubes and ventricose stipe, natural size.

cularly the rounded ends are highly chromophilous. In the upper part of the stipe the surface hyphae constitute a palisade; however, in the lower part, the surface often consists of 3–4 layers of overlapping, parallel hyphae, running longitudinally. There is no difference in the context of the pileus and stipe. In some sections hyphae in both regions have been seen forming chlamydospores.

Elevations in the lower surface of the pileus indicating the initiation of tube formation have been seen in another 24–48 hours, usually when the pileus is 0.6–1.5 cm broad. However, the stage of differentiation and the maturity of the hymenium does not necessarily correspond to the size of the carpophore. It appears that for the formation of the tubes the hyphae of the context, which formed the palisade, push the palisade outward in a spatulate fashion (Fig. 11). This palisade constitutes the hymenium from its very initiation, because, often at the earliest stage of its formation, some of the palisade cells can be seen bearing sterigmata, and often immature spores. The first elevation appears just above the union of the pileus to the stipe (Fig. 6). Further elevations then develop towards the margin of the pileus and also extend some distance down to the surface of the stipe (Fig. 8). In sections, the trama of the pileus near the hymenophore is seen to be very loose, causing the tubes to be detached easily from the context. The surface of the pileus is now covered with a cutis of 4–5 layers of parallel, overlapping, and repent hyphae (Fig. 10). Some of the palisade cells appear differentiated into cystidia and some into basidia. In some sections a triangular region in the context of the stipe, just below the union to the pileus, appears highly chromophilous and probably constitutes a region of growth for the development of the stipe. In the context of the pileus and stipe the hyphae partly lose their identity and seem imbedded in a gelatinous matrix. At this stage the color of the pileus is usually lemon yellow or lemon chrome, the margin incurved, the tubes pale yellow or almost colorless and soft, and the stipe is ventricose and buff yellow.

After the formation of the tubes, the further growth of the pileus and the maturation of the hymenium proceeds slowly under the cultural conditions, probably light, humidity, and other environmental factors playing an important role. Usually the hymenium is mature in 1–2 weeks and mature basidia and spores are always present, though very variable in number.

Description of the Mature Carpophore

Under cultural conditions the carpophores attain a maximum height of 4.5 cm with the pileus up to 2.5 cm broad, hemispheric (Fig. 4), lemon yellow, mustard yellow, or Pinard yellow in color, dried to apricot yellow or baryta yellow, tomentose, margin always incurved. Context mustard yellow blueing when cut, blue often disappearing after a few minutes. Hymenophore chrome yellow or Pinard yellow, becoming buffy citrine when fully mature, Mars yellow, antimony yellow, or sometimes mummy brown when dried, blueing when touched, tubes slightly decurrent, easily separating from the context of the pileus, 2–3 mm long. Pores irregular in size and shape, honey-combed or gyrodonlike (Fig. 17). Spore print brownish olive. Stipe often ventricose, usually 3.5 cm in length and 0.7 cm wide in the middle, tapering at the top

and bottom, mustard yellow or buff yellow, drying to mustard yellow or Naples yellow, smooth, tomentose, context often blueing when cut. Surface with a fertile palisade on the upper part, and parallel, overlapping cuticular hyphae on the lower, in some sections the fertile palisade extending almost to the base of the stipe. In rare cases the stipes grew to 6 cm long and were cylindric or tapering upwards. Odor none, taste mild.

Spores ovoid-ellipsoid to cylindric-oblong, pale yellow when young, melleous when mature, thin walled, 6–10×3–4.5 μ (Fig. 15). Basidia very variable in size and shape, often cut off with a basal septum, at the end of elongate hyphae, which could be traced into the trama of the hymenophore, some hyaline, but mostly dark yellow-brown, heavily granular. Cystidia variable in size and shape, some large with the upper portion broadly inflated or thin and ampullaceous (Figs. 12, 13, 14), hyaline or with granular, melleous contents.

Cuticular hyphae repent, narrow, 2–3 μ wide, hyaline or pale yellow. Trama of the tubes bilateral and divergent (Fig. 16), with the hyphae hyaline or pale yellow, showing no differentiation between mediostratum and lateral stratum.

Chemical Reactions

A bright yellow pigment from the tissue dissolves in 10% NH_4OH, KOH, or NaOH, but not in acids. *Gum guaiac*, negative; *phenol + aniline*, negative; *NH_4OH*, a weak greenish brown reaction on the surface of pileus and stipe; *KOH* and *NaOH*, Mars yellow to brick red on the surface of tubes and surface and context of pileus and stipe; *Formol*, negative.

Fruit body collected in Walker, La., U.S.A., DAOM 75195. Culture No. B-171-60. Carpophores from culture DAOM 75500.

Discussion

The collection in Louisiana consisted of one small fruit body and its identity was uncertain. In culture, however, the carpophores fit the description given by Singer (1947), and the description and illustration given by Kallenbach for *P. sulphureus* (Fr.) Sing. (1929). The gyrodonlike habit, the slightly decurrent tubes, the blueing context, the absence of a veil, and the ventricose stipe would place it in Singer's genus *Phlebopus*.

The developmental studies have shown that there is no indication of the presence of a universal veil at any stage of its development and therefore *P. sulphureus* has a gymnocarpic type of development, the hymenium being exposed from the time of its initiation until its full maturity.

Cases of gymnocarpic development in the Boletaceae have been reported by several authors and the subject was reviewed by Elrod and Snell (1940). A detailed description of gymnocarpic development was given by Zeller in his studies with *Boletellus zelleri* (Murr.) Sing., Snell & Dick (1914). In his account however, as in some other cases of gymnocarpy in the Boletaceae, it was found that the differentiation of stipe and pileus occurred on a cleavage plane which gave rise to an annular furrow and the hymenium was formed on the roof of this furrow. In the case of *P. sulphureus* no cleavage has been seen, the pileus being formed by a change in the direction of growth from

entirely vertical in the stipe, to partly radial, and the hymenophore being initiated after the lower surface of the pileus has been well developed. In some instances a fluffy white mycelium grew on the surface of the pileus, and this consisted of long and narrow hyphae, often arranged in loose strands. This, however, did not have the appearance of a veil and was never extended beyond the margin of the pileus. In one instance the pileus was covered with viscid fluid.

One of the most intriguing questions concerning the events leading to the fructification is what are the factor or factors controlling the development of the primordia towards a normal or an abnormal carpophore? It would appear that environmental factors have little effect since normal and abnormal carpophores often occur side by side in the same culture. It is interesting to note that the richer the medium the larger the number of abnormal fruit bodies, and the question of normality or abnormality might be due to metabolic processes and would need a biochemical investigation. It is not certain whether *P. sulphureus* is a mycorhizal former, although several members in the subfamily Boletoideae are known to form mycorhiza with frondose or coniferous trees. *P. sulphureus* has been reported as growing on wood and coniferous sawdust. Whether this is a case of more primitive saprophytic existence in the Boletaceae remains to be seen. It is certain, however, that members of the same subfamily and known mycorhizal partners produce primordia with an advanced stage of differentiation and it is hoped that the present success might lead to the production of the perfect state in more species in this group.

Acknowledgment

My sincere thanks go to Dr. J. W. Groves for his help in the identification of the carpophore and for reviewing this manuscript.

References

ELROD, R. P. and SNELL, W. H. 1940. Development of the carpophores of certain Boletaceae. Mycologia, **32**, 493–504.

KALLENBACH, E. 1929. Die Pilze Mitteleuropas, **1**, 57–60.

PANTIDOU, M. E. 1961. Cultural studies of Boletaceae. *Gyrodon merulioides* and four species of *Boletinus*. Can. J. Botany, **39**, this issue.

SINGER, R. 1947. The Boletineae of Florida with notes on extralimital species III. Am. Midland Naturalist, **37**, 3–4.

ZELLER, S. M. 1914. The development of the carpophores of *Ceriomyces zelleri*. Mycologia, **6**, 235–239.

A NEW SPECIES OF AGROPYRON FROM THE GREAT LAKES[1]

J. M. GILLETT AND H. A. SENN[2]

Abstract

The *Agropyron* population from the shores of the upper Great Lakes is considered to be morphologically distinct from that of the plains' species *A. dasystachyum* (Hook.) Scribn. and is here described as *A. psammophilum*. The geographical range is mapped and shown to be disjunct from that of the plains' species. *A. psammophilum* consistently occupies a sandy shore habitat. The species is tetraploid ($2n = 28$). Several explanations for the presence of empty pollen grains in some specimens of *A. psammophilum* are presented.

Introduction

The *Agropyron* population distributed about the sandy shores of Lakes Huron and Michigan has long been considered as a portion of the population of *A. dasystachyum* (Hook.) Scribn. of the plains' region. Hitchcock (1935) was aware of this population when he wrote: "In the form growing on the sandy shores of Lake Michigan the lemmas are villous, but villous forms occur in other parts of the range of the species." This statement was repeated in the subsequent edition of the *Manual of the Grasses of the United States* (Hitchcock and Chase 1951). Iltis, Reed, and Melcher (1960) recently referred to this population as: " . . . the endemic, as yet unnamed Great Lakes ecotype of *Agropyron dasystachyum* . . . ." In the herbarium of the Missouri Botanical Garden, Dr. G. B. Van Schaack evidently had been sufficiently aware of the population to remark on a specimen folder: "These are all on the *shores* of Lakes Huron and Michigan. Surely a n. sp. since *A. dasystachyum* was described from Saskatchewan. These cannot be of the same species as those growing in the Dakotas and west." Senn had been interested in this population for some years and dubbed it "*A. dasystachyum* Lake Form." This name appears on numerous herbarium labels annotated by him. This species was discussed briefly by Gillett in a report on *Agropyron* studies made at the American Institute of Biological Sciences meetings held at Stillwater, Oklahoma, in September, 1960.

Materials and Methods

A number of living plants and one collection of seed were gathered over a period of several years. Numerous herbarium specimens were made in various parts of the range of this and related species. Chromosome counts were secured from plants grown in the greenhouse and in pots out-of-doors. The method of accession of live material is similar to that given in Gillett and Senn (1960).

Herbarium material was borrowed from the Smithsonian Institution (US), the Gray Herbarium of Harvard University (GH), and the University of Wisconsin (WIS). Dr. J. H. Soper loaned some specimens and sent some

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records by letter from the University of Toronto (TRT). The collections at the Field Museum of Natural History (F) and at the Missouri Botanical Garden (MO) were examined during visits made there in the summer of 1960.

Results

TAXONOMY

Agropyron psammophilum sp. nov.

Perennis, radicibus fibrosis et rhizomatibus robustis. Culmi erecti, 4.5–9.5 (med. 6.0) dm alt. laeves striati in siccis, nodis 2–5, saepe purpureis. Folia sobolarum laxiter aggregata, caulinaria glaucissima 2–5 in culmo, inferiora ad 2.2 dm long., superiora ad 1.5–2.0 dm long., vaginibus laevibus, striatis in siccis, interdum basis purpureis, laminis rigidis et divaricatis, involutissimis in siccis, marginibus et in facie superna scabris, interdum puberulis. Ligula inconspicua, circa 0.4 mm long., glabra, marginibus laevibus. Auriculae 1.5 mm long., acutae. Spicae 7.5–25.5 (med. 15.0) cm long., exsertae, nodo inferiore spiculifero raro sterili aut rarius cum spica secundaria, internodis tenuibus et rigidis, marginibus laevibus vel scabridulis pubescentibus subter spiculis, inferioribus longioribus quam subsequentibus, spiculis singulis in nodo, rarius jugatis, 1.5–3.1 (med. 2.2) cm long., 9–18 (med. 11) in spica, floribus 4–8 in spicula. Glumae 0.7–1.4 (med. 0.9) cm long., pubescentia a sparsa densa, a basa sensim attenuatae, 3–6 jugis nervorum, ad summas acutae vel cum arista brevi. Lemmata 9–12 mm long., lanceolata, albovillosa, acuta, mucronata vel cum arista recta 1.0–1.5 cm long. Paleae breviores quam lemmatae, emarginatae, pubescentes, marginibus ciliatis. Antherae 4.5–6.0 mm long. Caryopsis 5–6 mm long.

Perennial, with fibrous roots and stout rhizomes. Culms erect, 4.5–9.5 (av. 6.0) dm tall, smooth, striate when dry, the nodes two to five, frequently purple. Offshoot leaves loosely tufted, the culm leaves two to five, very glaucous, the sheaths smooth, striate when dry, sometimes purple at the base, the blades stiff and spreading, up to 2.2 dm long at the base of the culm decreasing to 1.5–2.0 dm in the upper part, strongly involute when dry, the margins and upper surface scabrous, sometimes puberulent. Ligule inconspicuous, about 0.4 mm long, glabrous, the margin smooth. Auricles 1.5 mm long, acute. Spikes 7.5–25.5 (av. 15.0) cm long, exserted, the lower node bearing a spikelet, rarely sterile, more rarely with a secondary spike, the internodes thin and wiry, smooth or slightly scabrous-margined, pubescent immediately below the spikelets, the lower longer than the subsequent ones, the 9–18 (av. 11) spikelets borne singly at a node, rarely paired, 1.5–3.1 (av. 2.2) cm long with 4–8 florets. Glumes 0.7–1.4 (av. 0.9) cm long with 3–6 pairs of nerves, gradually attenuate from the base, acute or with a short awn, sparsely or densely short pubescent. Lemmas 9–12 mm long, lanceolate, white villous, acute, mucronate, or with a straight awn 1.0–1.5 cm long. Paleas slightly shorter than the lemmas, the apex notched, minutely pubescent, the margins ciliate. Anthers 4.5–6.0 mm long. Caryopsis 5–6 mm long.

Type specimen.—ONTARIO: Bruce County: *J. H. Soper* and *H. M. Dale 3964*, sandy dunes on beach, mouth of Pine River, 6 miles south of Kincardine, Lake Huron, June 19, 1948. Holotype DAO; isotypes GH, MO, TRT, US. This collection has been selected because it is distributed among several herbaria.

Other representative specimens.—CANADA: ONTARIO: Bruce County: Dorcas (Big) Bay, *Krotkov 8652* (DAO, TRT, US, WIS); Clark Point, *Soper 2809* (DAO, GH, TRT); 3 miles west of Amberley, *Senn 3213* (DAO); Saugeen, *Burgess in 1884* (DAO); Hopkins Bay, *Senn et al. 4954* (DAO); Southampton, *Dale 267* (TRT); Sauble Beach, *Dore 8984* (DAO, TRT, US). Manitoulin District: Manitoulin Island, Thomas Bay, *Soper & Grevatt 7208* (DAO, TRT); Providence Bay, *Pease & Ogden in 1935* (GH); Great Duck Island, Desert Point, *Soper & Heidenreich 8573* (DAO, TRT).

UNITED STATES: MICHIGAN: Benzie County: Frankfort, *Beal in 1888* (GH). Emmet County: Little Traverse Bay, *Camp & Camp 3298* (WIS); Petoskey, *Wheeler in 1879* (F). Huron County: Port Austin, *Morris A251* (GH, US). Iosco County: 2 miles north of Oscoda, *Loughbridge 4034* (DAO); Tawas Point, *Gilliman in 1872* (GH). Leelanau County: Sleeping Bear Dunes, *Gleason 234* (GH). Mackinac County: 4 miles west of Gros Cap, *Senn 5145* (DAO); Bois Blanch Island, *Iltis & Gates 2922* (US). Mackinac Island: *Eggert in 1878* (MO). North Manitou Island: *Wislizenus in 1891* (MO). Manitou Island: *Englemann in 1840* (MO). Schoolcraft County: Manistique, *Fernald & Pease 3136* (GH, US). St. Clair County: Port Huron, *Dodge in 1895* (GH, US). WISCONSIN: Door County: Washington Island, *Davis in 1931* (WIS); Whitefish Bay, *Schuette in 1883* (DAO, F, GH, US); Sevastopol, *Pohl 1110*, (WIS). Manitowoc County: Two Rivers, *Lapham s.n.* (GH, US, WIS). Ozaukee County: near Fort Washington, *Palmer 28856* (MO); Belgium, *Pohl 1157* (WIS). Sheboygan County: Sheboygan, *Wadmond s.n.* (GH, US); Holland, *Pohl 1005* (WIS); Terry Andreae Park, *Fuller 214* (WIS).

Agropyron psammophilum forma ***aristatum*** n.f.

Similis forma typica sed lemmatibus vel glumis rectis aristatis.

Type specimen.—MICHIGAN: St. Clair County: Shore of Lake Huron near Port Huron, *C. K. Dodge*, July 2, 1895. Holotype GH; isotype US.

Other representative specimens.—MICHIGAN: Emmet County: Sturgeon Bay, *Marshall 541* (DAO). Iosco County: Tawas Point, *Gilliman in 1872* (GH). St. Clair County: Fort Gratiot, *Pitcher s.n.* (GH).

A. psammophilum differs from *A. dasystachyum* as follows:

A. psammophilum	*A. dasystachyum*
Glumes: Tapering from the base; over ½ the length of the lemma; nerves prominent.	Sides parallel, tapering towards the tip, or blunt; usually much shorter than the lemmas to ½ their length; nerves inconspicuous.
Lemmas: Always pubescent	With variable pubescence or quite glabrous.
Awns: Straight; awned plants occasional in the population.	Divergent; awned and awnless forms in about equal frequency.
Spikes: 7.5–25.5 (av. 15.0) cm long; glaucous, usually with a bloom.	5.0–11.0 (av. 8.0) cm long; green, rarely glaucous.
Rachilla: Pubescent below the spikelets.	Glabrous below spikelets.
Habit: Loose, not sod-forming.	Sod-forming, occas. loose.
Pollen: Frequently empty.	Normal.
Habitat: Sandy beach dunes only.	Prairie, heavy soils, occasionally sand.

DISTRIBUTION

The distribution of *A. psammophilum* is given in Fig. 1. The species is restricted to the sandy shores of Lakes Huron and Michigan, but does not

extend to the southern shores of the latter. *Forma aristatum* has no distinct range within the population. The map has superimposed upon it the littoral surface currents (Anonymous 1892–94), because there appears to be some correlation between the direction of flow and the distribution of the species. Because it is a rhizomatous plant of sand dunes, this correlation rather indicates that it may be dispersed by wave action. The existence of these currents may explain why the species does not extend further south along the sandy shores of Lake Michigan and why the species does not extend into Lake Superior.

FIG. 1. Distribution of *Agropyron psammophilum* Gillett & Senn. (Arrows indicate the direction of littoral currents.)

However, with respect to the occurrence of *A. psammophilum* on the shores of Lake Superior, we have seen two collections by A. O. Farwell, Nos. 794 and 794a, both from "Keweenaw Peninsula, Michigan," in the Smithsonian Institution herbarium. No. *794* is dated August 1890; No. *794a* is dated June 1895. The labels are so identical in information and handwriting that we suspect they were written together at a later date. We have not seen any other specimen from the shores of Lake Superior, so we have disregarded these specimens until substantiated by other collections. According to McVaugh (1953), Farwell's

numbering system was complex, so we cannot attempt to interpret them or relate them to other collections to get more specific information of locality.

Dodge (1914) described the distribution of *A. dasystachyum* in his "Annotated list of flowering plants and ferns of Point Pelee, Ontario," as "In sandy ground near shore on Pelee Island but nowhere abundant." No specimen has been found to corroborate this statement and we are unable to determine whether or not it is an introduction of *A. dasystachyum* of the plains. Core (1948) added nothing to the statement, merely citing Dodge. Krotkov (1940) reported our species under *A. dasystachyum*, "In sand at Big Bay and Stokes Bay." Big Bay is now called Dorcas Bay.

SOMATIC CHROMOSOME NUMBER

Chromosome counts of $2n = 28$ for 13 collections from various parts of the range of *A. psammophilum* have been recorded. These counts are listed below in geographical sequence. All determinations have been made by W. M. Bowden of this Institute.

CANADA: ONTARIO: Bruce County: Clark Point, Cytological Accession No. 587 (*Dore 8903*); Dorcas Bay, Cyt. No. 864 (*Senn 5128*), Cyt. No. 865 (*Senn 5129*), Cyt. No. 866 (*Senn et al. 5130*); near Tobermory, Cyt. No. 868 (*Senn et al. 5132*); 2 miles south of Sauble Beach, Cyt. No. 417 (*Senn 3218*); 3 miles south of Amberley, Cyt. No. 415 (*Senn 3213*). Manitoulin District: Manitoulin Island, Hensley Bay, Cyt. No. 1507 (*Senn 6105*); Providence Bay, Cyt. No. 1091 (*Senn s.n.*).

UNITED STATES: MICHIGAN: Emmet County: Wilderness Park, Cyt. No. 936 (*Senn 5156*). Mackinac County: 38 miles east of St. Ignace, Cyt. No. 556 *Bibbey s.n.*); 7 miles east of Naubinway, Cyt. No. 1677 (*Senn 6270*). Schoolcraft County: 6 miles west of Manistique, Cyt. No. 1676 (*Senn 6266*).

All of the above voucher specimens are deposited in the herbarium of the Plant Research Institute at Ottawa.

INTERSPECIFIC HYBRIDS

We have detected two cases of natural hybrids between *A. psammophilum* and *A. trachycaulum* (Link) Malte. These are putative hybrids in the sense that the two parent species occur in adjacent habitats. The hybrids combine the parental characters, but have yet to be produced experimentally.

The first case concerns three collections from Dorcas Bay, Bruce County, Ontario, made by Senn on July 21, 1949. *Senn 4925* is *A. psammophilum* from the beach sand habitat and is characteristically very glaucous and rhizomatous; its anthers are large and contain 95% good pollen. *Senn 4926* is *A. trachycaulum* from the edge of woods above the beach and is characteristically green and nonrhizomatous; its anthers are small and the pollen is good. *Senn 4927* is the hybrid, growing in sand at the extreme margin of the woods; it is slightly glaucous, rather intermediate in spike morphology, and bears vigorous rhizomes. The medium-sized anthers are without good pollen.

The second case concerns two collections made by W. G. Dore from the same area, Dorcas Bay, July 27, 1948. One accessioned as Cyt. No. 588 (*Dore 8993*, $2n = 28$) has no good pollen. The spikes are very long and similar to *A. trachycaulum*. The plant is rhizomatous and glaucous. The other collection,

Cyt. No. 589 (*Dore 9004*, $2n = 28$), from the same locality has withered anthers, the pollen is 0% good, and the specimen exhibits intermediate morphology between *A. trachycaulum* and *A. psammophilum*. The latter species represented by *Dore 900* was gathered at the same place but the other parent was not sought because hybridity was not suspected at the time the specimens were collected.

Of considerable interest was a meiotic study made on the two Dore collections. Moore found in Cyt. No. 588 that pairing was irregular; the average pairing was $0.31^{I}+11.7^{II}+0.08^{III}+0.54^{IV}+0.8^{VI}$. The average chiasmata frequency per cell was 27.1 and pollen was aborted. In Cyt. No. 589 the average pairing was $0.44^{I}+12.2^{II}+0.22^{III}+0.44^{IV}+0.11^{VI}$ and the average chiasmata frequency per cell was 26.6. Again no good pollen was found.

The decision that the Dore specimens were hybrids was first made on the basis of intermediate morphology and pollen sterility, and the above information made available later served to corroborate this opinion.

POLLEN AND SEED SET

Much of the herbarium material of *A. psammophilum* has shown a high incidence of empty pollen grains. A 2% solution of alcoholic iodine in potassium iodide has been employed as a stain both with herbarium material of this species and with the western plains' species *A. dasystachyum*. Other stains such as cotton blue have been employed with the same result but the iodine stain is faster in its action. In these tests *A. dasystachyum* displayed a high degree of pollen fertility in all specimens examined. *A. psammophilum*, however, has almost completely empty grains in much of the material.

The following collections had completely aborted pollen: Cyt. No. 936S2 (*Senn 5156*), pollen 0% good but the ovary appears to be developing normally; Cyt. No. 1677 (*Senn 6270*), pollen 0% good in extremely small quantity, ergot present; Cyt. No. 866 (*Senn 5130*), pollen 0%, sparse; Cyt. No. 866 (*Senn 5139*), 0% but 96% good when grown in the greenhouse; Cyt. No. 864 (*Senn 5128*), pollen 0%, anthers poorly developed, 3.0 mm long; *Senn 4791*, anthers bad, no pollen, no germination of seed, only basal florets with good grains (cytologist's remark on sheet); *Dore 8903*, pollen 0%, anthers 5.0 mm; *Senn 3213*, pollen 0%, anthers 3.3 mm, a few good seeds set; *Senn et al. 4953*, anthers hard, 2.25 mm long, pollen 0%; Cyt. No. 868 (*Senn et al. 5132*), anthers 3.0 mm, pollen 5%, 0% on a second sheet; *Senn 3218*, anthers 5.0 mm, pollen 0%.

On the other hand, Ehlers 513 (US) had 95% good pollen; *Krotkov 8652* (WIS), 93% good; *Krotkov 7062* (US), 99%; *Soper & Dale 3964*, 98.2%; *Soper & Grevatt 7208*, 98%; and *Pease & Ogden in 1935*, 98% good. However, *Dodge in 1895* had pollen over 50% good. These are selected representative specimens.

Discussion

The specialized habitat and morphological differences indicate that this population should be considered as a separate species from *A. dasystachyum* of the plains, the only other species to which it may be related. Geographically *A. psammophilum* is separated by several hundreds of miles from the natural range of the plains' species. The latter occurs only as an introduction

in railway yards and similar disturbed habitats in the Great Lakes region.

We offer no explanation for the occurrence of empty pollen grains in numerous specimens of what appear to be typical *A. psammophilum*. In the past the occurrence of aborted pollen has always been indicative of the hybrid nature of material under examination. For example, tests on pollen of the plains' species *A. dasystachyum* (Hook.) Scribn. using the same iodine stain technique indicate that pollen is consistently good. If the pollen is abortive, then we immediately suspect that the specimen is a hybrid. In the case of *A. psammophilum* we have found only two putative hybrids with *A. trachycaulum*. These hybrids were recognized by the combination of intermediate morphology and abortive pollen, supported by the observation that meiosis was irregular. There has been no correlation found in other specimens of *A. psammophilum* having aborted pollen with any atypical morphology.

Agropyron psammophilum may be included among the other tetraploid North American species as *A. dasystachyum* (Hook.) Scribn. and *A. trachycaulum* (Link) Malte. These species possess the ability to cross occasionally and hence provide a mechanism for the potential evolution of further populations.

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References

ANONYMOUS, 1892–94. United States Dept. of Agric. Weather Bureau. Surface Currents of the Great Lakes. In Report of the Select Committee of the Ontario Legislative on Lake Levels of the Great Lakes. Baptist Johnson, Q.P. 1953.

CORE, E. L. 1948. The flora of the Erie Islands. Ohio State Univ. Contr. No. 9.

DODGE, C. K. 1914. Annotated list of flowering plants and ferns of Point Pelee, Ontario and neighboring districts. Can. Geol. Surv. Mem. 54.

GILLETT, J. M. and SENN, H. A. 1960. Cytotaxonomy and infraspecific variation of *Agropyron smithii* Rydb. Can. J. Botany, **38**, 747–760.

HITCHCOCK, A. S. 1935. Manual of the grasses of the United States. U.S. Dept. Agr. Misc. Publ. 200.

HITCHCOCK, A. S. and CHASE, A. 1951. Manual of the grasses of the United States. 2nd ed. U.S. Dept. Agr. Misc. Publ. 200.

ILTIS, H. H., REED, J., and MELCHER, T. 1960. *Elymus arenarius* and *Diarrhena americana* in Wisconsin. Rhod. **62**, 199–201.

KROTKOV, P. V. 1940. Botanical explorations in the Bruce Peninsula, Ontario. Trans. Roy. Can. Inst. **23**, 3–65.

MCVAUGH, R. 1953. Farwelliana. Cranbrook Inst. Sci. Bull. **34**.

BRYOPHYTES FROM THE EDMONTON REGION, ALBERTA[1]

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Abstract

For an area in south-central Alberta, 159 bryophytic taxa are reported. Approximately, two-thirds of these are new records for the area and 19 are apparently new to Alberta. Some of the more significant records for Alberta are *Sphagnum robustum, Aloina rigida, Brachythecium acutum, Calliergon trifarium, Leskea obscura, Haplocladium microphyllum, Thuidium philibertii, Entodon cladorrhizans,* and *E. orthocarpus,* the latter a rare species in North America. Ecological notes are included for most of the species. Moss synusiae of wet meadow, wet thicket, *Sphagnum* bog, bog forest, and marl bog are described and the main hydroseres are indicated.

This paper presents (*a*) a catalogue of the mosses and liverworts collected within a radius of about 100 miles of Edmonton, with ecological notes on the species, and (*b*) a brief account of certain moss communities of the region. The area of the report is approximately 32,000 square miles, which is less than one-seventh of the total area of Alberta.

The area is largely within the Boreal Forest Zone but, in the western part, there is Boreal–Cordilleran Forest and, in the southeast, Parkland Prairie. Of course, much of the region has been brought under cultivation or otherwise modified by man's activities. The physical features, climate, and soils of the region are described in earlier papers (5, 6, 7, 10, 11, 12) which also record some of the chief moss species in spruce, pine, poplar, bog, and other plant communities.

Actually, relatively few of the bryophytes of the Edmonton region have been recorded. A recent survey of the literature by Bird (2) reveals that only 43 moss species have been reported for the area. The present paper, while greatly extending the bryophytic record, must be regarded as only the first phase of a comprehensive investigation.

We are greatly indebted to Dr. C. D. Bird, who has recently initiated a study of Alberta bryophytes, for his encouragement and assistance, also for the privilege of including some of his own Edmonton records in this paper. To those taxonomists who have helped with species determinations we are very grateful. Some of our earlier collections were examined by H. N. Dixon, A. J. Grout, A. H. Brinkman, A. L. Andrews, and G. H. Conklin. Later collections were determined by A. L. Andrews, W. C. Steere, R. F. Cain, F. J. Hermann, Margaret Fulford, and H. Crum. To Dr. Crum, who has reported on quite a large proportion of our material, we owe a special debt of gratitude.

In the following catalogue, collection numbers and geographical locations are included only for species that are apparently rare in the region or that have been collected only a few times. The collection numbers are preceded by the surname initial of the collector or, exceptionally, by the collector's name. The

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bryophytic nomenclature is mainly that of a recent catalogue by Bird (2), following *Index Muscorum* where possible. For vascular plants the nomenclature follows Moss, *Flora of Alberta* (Toronto, 1959). Nearly all of the collections used as the basis for this paper are represented in the herbarium of the University of Alberta, and many have been sent to the National Museum of Canada.

Catalogue of Mosses and Liverworts

HEPATICAE

The region is not rich in liverworts, a fact readily explained in terms of the prevailing continental climate. Moreover, little collecting seems to have been done. Brinkman may actually have seen some material from the Edmonton region but this is not indicated in his papers (3, 4). The following list should be regarded as far from complete for the hepatics of the region.

Blepharostoma trichophyllum (L.) Dumort.
M6871c, banks and rotting wood, bog forest, and M6873, *Sphagnum* bog, Peers, 100 miles west of Edmonton.

Calypogeia muelleriana (Schiffn.) K. Muell.
M11023a, on *Sphagnum* mound, in spruce–poplar–alder woods, Bruderheim, 30 miles northeast of Edmonton. Placed here rather than in *C. neesiana* because the underleaves are lobed.

C. trichomanis (L.) Corda
M11038b, with *Lepidozia reptans*, on decayed *Sphagnum* mound, spruce–poplar woods, Bruderheim.

Cephalozia media Lindb.
M6871b, with *Drepanocladus revolvens* and *Blepharostoma*, Peers. M11023b, with *Calypogeia* and other hepatics on *Sphagnum*, spruce–poplar–alder woods, Bruderheim.

Conocephalum conicum (L.) Dumort.
M9224, wet bank, poplar–spruce woods, Calling Lake, 130 miles north of Edmonton. Poplar–spruce woods, river valley, Edmonton, but collections not preserved.

Jamesoniella autumnalis (DC.) Steph.
M2036, with mosses and *Ptilidium ciliare*, coniferous woods, Fork Lake, 80 miles northeast of Edmonton.

Lepidozia reptans (L.) Dumort.
M11038a, on decayed *Sphagnum* mound, spruce–poplar woods, Bruderheim.

Lophocolea minor Nees
M4169, on decayed spruce stump, mixed woods, Edmonton.

Lophozia incisa (Schrad.) Dumort.
M6874a, with *L. porphyroleuca*, *Cephalozia*, and *Riccardia*, on rotting wood, spruce bog forest, Peers. M8952a, with other liverworts and mosses, tamarack – black spruce bog, Edmonton.

L. porphyroleuca (Nees) Schiffn.
M6874b, with *L. incisa* and other hepatics, on rotting wood, bog forest, Peers.

Marchantia polymorpha L.
Common throughout the region, especially in recently burnt woods and swamps, where it grows as a pioneer on moist denuded ground.

Mylia anomala (Hook.) S. F. Gray
Common on *Sphagnum*, in muskegs. M1752, spruce bog, Wetaskiwin, 40 miles south of Edmonton. M8985, open bog, Edmonton.

Pellia fabbroniana Raddi
M4167, wet, calcareous (tufa) bank, White Mud Creek, Edmonton.

Preissia quadrata (Scop.) Nees
T1760, on shaded, clay bank of river, Fort Saskatchewan.

Ptilidium ciliare (L.) Nees
Common on rotting stumps and logs, and on mossy banks, in various kinds of moist woods. M1964, Cooking Lake, and M7819, Winterburn, both near Edmonton.

P. pulcherrimum (Web.) Hamp.
M4168, on decayed spruce stump, mixed woods, Edmonton. T9993a, on the ground, black spruce swamp, Elk Island Park. Intergrades in the region with the previous species.

Reboulia hemisphaerica (L.) Raddi
M2182, on shaded, wet, silt–clay bank of river, Edmonton.

Riccardia latifrons Lindb.
M6875c, with several other hepatics on rotting wood, spruce bog forest, Peers.

R. pinguis (L.) S. F. Gray
M2087, on mossy banks and tufa mounds, wet with water from springs, White Mud Creek, Edmonton.

Ricciocarpus natans (L.) Corda
Edmonton area, but seen only three times in a period of 40 years; M636, on water of a shallow pond, where abundant and mixed with *Lemna minor*, 15 miles southeast of Edmonton.

SPHAGNALES

A preliminary report on Alberta *Sphagnum* has already been produced in the form of an unpublished thesis (12). The following list is based on the earlier report, but with much of the collection data omitted and with a few amendments. Ecological aspects of the more prevalent species are considered in a later section of this paper.

Sphagnum capillaceum (Weiss.) Schrank
(*S. acutifolium*; *S. nemoreum*)
Probably the most common species of the genus in the region; it occurs not only in bogs and bog forest but also occasionally on the floor of wet poplar and white spruce woods.

S. capillaceum var. *tenellum* (Schimp.) Andrews
(*S. rubellum*)
Widespread in bogs, occasional in white spruce forest.

S. centrale C. Jens.
Occurs rather sporadically in various kinds of bogs and swamps. T10576, tamarack swamp, Pigeon Lake, 35 miles southwest of Edmonton. M9519, wetter part of muskeg, Jarvie, 70 miles north of Edmonton. The first report of this species for the region, at least under the name *S. centrale*. Our earlier collections, understandingly reported (12) as *S. palustre* L., were determined before Andrews (1) had decided to reinstate *S. centrale* as an independent species distinct from *S. palustre* and S. *magellanicum*.

S. fuscum (Schimp.) Klinggr.
Widespread, abundant in drier parts of open bogs and bog forests.

S. girgensohnii Russow
Not common. M1952, spruce–poplar woods, Woodbend, near Edmonton. M2619, shaded place in bog forest, west of Lacombe.

S. magellanicum Brid.
Fairly common, especially in rapidly growing bogs.

S. recurvum P. Beauv.
A common species in wetter parts of developing bogs. The report (12) of *S. cuspidatum* Ehrh. for the region was based on a poor collection, M1639, which is probably *S. recurvum*.

S. squarrosum Crome
Infrequent. M6774, in fenlike vegetation, moat of muskeg, Seba, 50 miles west of Edmonton. T10596, with willows, *Aulacomnium* and *Drepanocladus*, marsh, Pigeon Lake.

S. robustum (Russow) Roell.
Apparently rare. C. D. Bird 5396, in a *Picea–Ledum* mature bog, St. Joseph Lake, 25 miles southeast of Edmonton, Sept. 11, 1960. This appears to be a new record for the region.

S. subsecundum Nees
Widespread, but confined to very wet parts of bogs; such habitats are rather rare and quite localized in the region.

S. tenerum Sull. & Lesq.
Rather infrequent and usually in wet thickets bordering muskegs or forming mats at the edge of a *Drepanocladus* bog. This species is recognized with some hestitation, for it seems very close to *S. capillaceum*.

S. teres (Schimp.) Aongstr.
Not common, though represented by seven collections from the region. Wet *Drepanocladus–Carex* bogs and low parts of *Picea–Ledum* muskeg.

S. warnstorfii Russow
(*S. warnstorfianum*)
Widespread and usually localized. Commonly in bog forest, occasionally in low, white spruce woods. Common associates are *S. capillaceum*, *S. fuscum*, *Aulacomnium palustre*, *Drepanocladus aduncus*, *Polytrichum* spp., and 'feather mosses'.

Bryales

Abietinella abietina (Hedw.) Fleisch.
(*Thuidium abietinum*)
A common moss of poplar and white spruce woodlands, usually on relatively dry ground, sometimes on slopes and banks, often mixed with *Hylocomium*, *Pleurozium*, and *Brachythecium salebrosum*.

Aloina brevirostris (Hook. & Grev.) Kindb.
Collection of D. S. Correll, Edmonton, in 1947, reported by Steere (13).

A. rigida (Hedw.) Limpr.
Found in only one area. C. D. Bird 4990, bare ground, face of high river bank, Fort Saskatchewan, 12 miles northeast of Edmonton. A new Alberta record.

Amblyodon dealbatus (Hedw.) P. Beauv.
M11078a, moist bank, edge of calcareous pool, with *Distichium capillaceum* and *Campylium stellatum*, marl bog, about 12 miles northwest of Edmonton.

Amblystegium juratzkanum Schimp.
Often not readily distinguished from *A. serpens*, with which it seems to intergrade. T9751, on rotted wood, under poplar, Fort Saskatchewan. T11058, on very wet wooden flume, the moss thriving in shallow running water, Fort Saskatchewan.

A. serpens (Hedw.) B. S. G.
Common on damp ground, banks, and rotted wood, intermixed with *Eurhynchium* spp., *Brachythecium* spp., *Haplocladium microphyllum*, *Ceratodon purpureus*, and *Mnium* spp.

Anisothecium schreberianum (Hedw.) Dix. var. *elatum* (Schimp.) Wijk. & Marg.
(*Dicranella schreberi* var. *elata*)
T9983, in wet road ditch, Pigeon Lake. A rather rare moss.

A. varium (Hedw.) Mitt.
(*Dicranella varia*)
T10134, sloping face of river bank, near Fort Saskatchewan. T10831, in shade of poplar, on river bank, near Fort Saskatchewan. An early record by Macoun (8): mud banks along the Athabasca River, Fort Assiniboine, 70 miles northwest of Edmonton.

Aulacomnium palustre (Hedw.) Schwaegr.
A very common species of swamps, bogs, marshes, and wet woods. Associated mosses include *Sphagnum* spp., *Polytrichum* spp. *Drepanocladus aduncus, Helodium blandowii, Tomenthypnum, Hylocomium,* and *Pleurozium.* A report (10) of *A. turgidum* (Wahlenb.) Schwaegr. is based on a misidentification of M789, a collection now placed as *A. palustre.*

Barbula acuta (Brid.) Brid.
T10137, steep face of river bank, on an island in the North Saskatchewan River, near Fort Saskatchewan.

B. fallax Hedw.
M12335, silt bank, near river, Edmonton.

Brachythecium acutum (Mitt.) Sull.
T9639, on ground, heavy poplar woods, Fort Saskatchewan. M12308, disturbed tamarack bog, 15 miles northeast of Fort Saskatchewan. M11072, with *Campylium chrysophyllum,* forming dense mats on ground and as "stockings" at bases of small tamarack trees, bog forest, 10 miles southeast of Edmonton. First report for Alberta.

B. campestre B.S.G.
T10081, under poplars, on sandy soil, edge of old road, slope above river bank, Fort Saskatchewan. First report for Alberta.

B. plumosum (Hedw.) B.S.G.
(*B. flagellare*)
T9935, on low ground, under willows and spruce, creek valley, Fort Saskatchewan.

B. salebrosum (Web. & Mohr) B.S.G.
Perhaps the most abundant woodland moss in the region, found on fairly dry shaded banks as well as on moist low ground, sometimes growing as a "stocking" around the foot of poplars. Associated mosses include *Pylaisia* spp., *Mnium* spp., *Hylocomium, Pleurozium, Haplocladium microphyllum, Abietinella abietina,* and *Eurhynchium pulchellum.* Earlier reports (10) of *B. oxycladon* (Brid.) J. & S. should be referred here.

Bryoerythrophyllum recurvirostre (Hedw.) Chen
(*Didymodon recurvirostre*)
A common moss of various habitats including ground under poplar trees, moist banks in woods, floor of tamarack bog, and face of river bank.

Bryum argenteum Hedw.
T10123, on ground, top edge of clay river bank, near Fort Saskatchewan. T10234, ground, under poplar, river flat, Fort Saskatchewan. C. D. Bird 3946, north-facing roadside bank, near Pine Lake, southeast of Red Deer.

B. biddlecomiae Aust.
M11053, wet ground, near spring outlet, iron springs area, White Mud Creek, Edmonton.

B. caespiticium L. ex Hedw.
M2004, balsam poplar stand, Ponoka, 60 miles south of Edmonton. M11055, moist bank, mixed woods, near Edmonton.

B. capillare L. ex Hedw.
T10082, T10129, in shade of poplar, river flats, Fort Saskatchewan.

B. cirratum Hopp. & Hornsch.
M11022, moist ground, spruce–poplar–alder woods, Bruderheim. M11029c, burned-over tamarack swamp, north of Bruderheim.

B. creberrimum Tayl.
(*B. cuspidatum*)
M1817, floor of poplar woods, near Edmonton. T9791, spruce swamp, Pigeon Lake.

B. pallens Sw.
M11049, marl bog, White Mud Creek, Edmonton.

B. pseudotriquetrum (Hedw.) Schwaegr.
T9894, tamarack bog, north of Fort Saskatchewan. M11042, with *Scorpidium*, calcareous pool, marl bog, Acheson, near Edmonton. M11080, with *Campylium stellatum*, edge of calcareous pool, marl bog, 12 miles northwest of Edmonton.

B. turbinatum (Hedw.) Turn.
M783, moist ground, poplar grove, Ellerslie, near Edmonton.

B. uliginosum (Brid.) B.S.G.
(*B. cernuum*)
M11035, with *Leptobryum pyriforme* and other mosses, spruce–poplar–alder woods, near Bruderheim. M11051, moist tufa mounds, iron springs, White Mud Creek, Edmonton.

Calliergon cordifolium (Hedw.) Kindb.
Reported (7) as *Hypnum cordifolium*, but no voucher specimens have been found for checking. Pools in bog forest, west of Edmonton.

C. giganteum (Schimp.) Kindb.
(*Acrocladium giganteum*)
M2085, M4164, T1212, in large pool fed by iron springs, White Mud Creek, Edmonton.

C. sarmentosum (Wahlenb.) Kindb.
M425b, edge of depression, somewhat boggy area, Cooking Lake, 15 miles southeast of Edmonton. M12352b, moat of bog, Boag Lake, near Edmonton. New Alberta record.

C. trifarium (Web. & Mohr) Kindb.
M2808, with *Drepanocladus aduncus*, edge of lake in a large wet bog, near Fawcett, 90 miles north of Edmonton. First report for Alberta.

Campylium chrysophyllum (Brid.) J. Lange
On various substrata in woodlands. T9815, on clay top of river bank, under poplars, near Fort Saskatchewan. M11071, as dense mats, on ground and "stockings" at bases of small tamarack trees, bog forest, near Edmonton. M11095b, on rotting log, poplar–spruce woods, Edmonton.

C. hispidulum (Brid.) Mitt.
M820, floor of aspen woods, Elk Island Park, 25 miles east of Edmonton.

C. radicale (Beauv.) Grout
M1401, with *Calamagrostis canadensis*, on floor of a very wet meadow, Cooking Lake, southeast of Edmonton. First report for Alberta.

C. stellatum (Hedw.) C. Jens.
Wet places in calcareous bogs. M11044, Acheson, near Edmonton. M11063d, Calahoo, 25 miles northwest of Edmonton.

Catoscopium nigritum (Hedw.) Brid.
M11046, with *Scorpidium scorpioides*, calcareous pools, boggy area, Acheson, near Edmonton. M12276, edge of pond, marl bog, Heatherdown, 20 miles northwest of Edmonton.

Ceratodon purpureus (Hedw.) Brid.
Very common, characterizing such habitats as bare ground in prairie grassland, sand hills, rotting wood in various kinds of woodland, ground in burned-over woods and bogs, wooden shingles on roofs.

Climacium americanum Brid.
M6725, white spruce grove, near Edmonton. M11039, low spruce–poplar woods, north of Bruderheim.

C. dendroides (Hedw.) Web. & Mohr
Apparently more prevalent than the last species. T12, wet edge of pool, creek valley, Fort Saskatchewan. T9919, wet ground, under willows, Pigeon Lake. M786, poplar grove, near Edmonton. Common associates are *Drepanocladus aduncus*, *Mnium* spp., *Aulacomnium palustre*, *Thuidium philibertii*, *Brachythecium salebrosum*.

Cratoneuron filicinum (Hedw.) Spruc.
M765, M11058, borders of pools and pond, fed by water from iron springs, White Mud Creek, Edmonton. The report (5) of *C. commutatum* (Hedw.) Roth. for the region is apparently based on a misidentification.

Desmatodon cernuus (Hueb.) B.S.G.
Reported by Macoun (9) on earth, Pembina River, northwest of Edmonton.

D. latifolius (Hedw.) Brid.
On earth, Edmonton, according to Macoun (9).

Dicranum bergeri Bland.
The most common *Dicranum* in the region; bogs and swamps, also on the floor of low poplar and white spruce woods.

D. bonjeani De Not.
M1901a, poplar grove, Woodbend, near Edmonton. T9914, on rotted stump, low white spruce woods, north of Bruderheim.

D. elongatum Schleich.
M1716, on decayed log, white spruce grove, 10 miles southeast of Edmonton.

D. elongatum subsp. *groenlandicum* (Brid.) C. Jens.
(*D. groenlandicum*)
M6883, in *Betula–Larix–Drepanocladus* bog, near Peers.

D. flagellare Hedw.
T9896a, black spruce bog, Pigeon Lake. T10008, low white spruce woods, near Fort Saskatchewan.

D. fragilifolium Lindb.
T9791, black spruce bog, Pigeon Lake. T9884, on rotted stump, low white spruce woods, north of Bruderheim.

D. fuscescens Turn.
M6870, on dry hummock, black spruce forest, Peers. T9951b, on rotted stump, low white spruce woods, Battle Lake, 50 miles southwest of Edmonton.

D. rugosum (Hoffm.) Brid.
T9981, low white spruce woods, Pigeon Lake. T9990, black spruce bog, Elk Island Park. M8687, pine woods, Whitecourt, 90 miles northwest of Edmonton.

D. scoparium (L.) Hedw.
M819b, floor of aspen woods, Lamont, 30 miles northeast of Edmonton.

D. strictum Schleich.
T9951, on ground and rotted wood, low spruce woods, Battle Lake. T9970, rotted stump, low spruce woods, Pigeon Lake.

Distichium capillaceum (Hedw.) B.S.G.
A common moss of forests, usually in moderately moist uplands, but also in bogs. Associated species include *Mnium cuspidatum*, *Brachythecium salebrosum*, *Abietinella abietina*, *Ditrichum flexicaule*, *Entodon* spp., *Drepanocladus uncinatus*, *Timmia megapolitana*, *Bryoerythrophyllum recurvirostre*, *Bryum* spp.

D. inclinatum (Hedw.) B.S.G.
M11054a, with *D. capillaceum*, tufa mound, White Mud Creek, Edmonton.

Ditrichum flexicaule (Schwaegr.) Hamp.
Often mixed with *Distichium capillaceum* and *Abietinella abietina*. T1607, under heavy poplar, near Fort Saskatchewan. T9744a, T10123, under white spruce, slope above river bank, Fort Saskatchewan.

Drepanocladus aduncus (Hedw.) Warnst.
Included here is *D. sendtneri* (Schimp.) Warnst., in accordance with a recent revision by Wynne (16). Common in bogs where it often forms thick peat, also on low wet ground with willows, poplar, spruce, tamarack. M563, forming mats, wet sedge bog, near Edmonton. T9768, edge of shaded pond, Fort Saskatchewan. T9982, on low ground under willows, Pigeon Lake.

D. aduncus var. *kneiffii* (B.S.G.) Moenk.
T9769, in shallow water, creek valley, Fort Saskatchewan.
M12356, wet meadow adjoining slough, 10 miles east of Edmonton.

D. aduncus var. *polycarpus* (Bland.) Warnst.
T1611b, wet bank of creek, Fort Saskatchewan. T9894, M11026, low wet ground, tamarack bog, north of Bruderheim.

D. aduncus var. *polycarpus* f. *gracilescens* (B.S.G.) Moenk.
M12361, *Calamagrostis* marsh, 10 miles west of Edmonton.
T1611b, creek bank, T11247, on a rotted tree on ground, both at Fort Saskatchewan.

D. fluitans (Hedw.) Warnst.
M2079, pool fed by springs, river valley, Edmonton.

D. revolvens (Turn.) Warnst.
M11063b, in calcareous pools, *Larix–Picea* marl bog, Calahoo, 15 miles northwest of Edmonton. C. D. Bird 4306, wet edge of marl bog, near Heatherdown. Some of our collections appear to intergrade with the following variety.

D. revolvens var. *intermedius* (Lindb.) Richs. & Wall.
(*D. intermedius*)
M9184, peat-forming, in a bog, south of Hondo, about 100 miles north of Edmonton.

D. uncinatus (Hedw.) Warnst.
A very common species, often on relatively dry banks or rotting logs in various kinds of woodland, occasionally on damp ground in unsheltered places.

D. vernicosus (Lindb.) Warnst.
A common peat-forming moss. M581, in a floating mat, with *Carex* spp. and *Menyanthes*, wet depression, near Edmonton. M2796, with *Aulacomnium palustre*, on wet floor of a sedge bog, Nestow, 60 miles north of Edmonton. M6880, comprising a zone bordering a bog lake, near Peers.

Entodon cladorrhizans (Hedw.) C. Muell.
M10985, M10988, shaded bank, poplar–spruce–birch woods, river valley, Edmonton. New Alberta record.

E. orthocarpus (LaPyl.) Lindb.
T1609, T9669, forming extensive thick mats, associated with *Abietinella abietina* and several other mosses, under white spruce, on a steep slope above the south bank of river, near Fort Saskatchewan. M10981, M12315, on shaded moist banks, spruce–poplar–birch woods, river valley, Edmonton. T1609, July 9, 1939, appears to be the first collection for Alberta and for continental Canada. The species is known for Newfoundland and a few locations in the United States.

Eurhynchium diversifolium (Schleich.) B.S.G.
Often not readily distinguished from the next species. T9656, on shaded roadside bank, Fort Saskatchewan. T9799, under poplar trees, lake bank, Battle Lake.

E. pulchellum (Hedw.) Jenn.
(*E. strigosum*)
A common species, on shaded moist banks, rotting wood, and bases of trees, often associated with *Pylaisia polyantha*, *Brachythecium salebrosum*, and *Haplocladium microphyllum*.

Fissidens viridulus (Web. & Mohr) Wahlenb. var *tamarindifolius* (Brid.) Grout
Reported (9) from Lac la Nonne, 40 miles northwest of Edmonton.

Fontinalis duriaei Schimp.
T9781, in shallow water, under heavy poplars, beside Battle Lake. T9807, in water, edge of stream, Pigeon Lake.

F. hypnoides C. J. Hartm.
Collections of J. Macoun from the Battle River region, about 50 miles south of Edmonton, are reported by Welch (15). A report (9) of *F. novae-angliae* Sulliv. for brooks north of Edmonton is probably based on a misidentification, as Welch (15) has no record of this species west of Winnipeg.

Funaria hygrometrica Hedw.
Very common as a "fireweed" on ashy substrata of burnt forest, bogs, and brush, also found on other bare areas and associated with various species.

Haplocladium microphyllum (Sw.) Broth.
(*Thuidium microphyllum*)
Prevalent on hummocks and wood, usually obscured by other mosses. T9864, rotted log, under white spruce, Fort Saskatchewan. M12330, moist rotting wood, poplar–spruce stand, Edmonton. First report for Alberta. Some of our material is close to subspecies *virginianum* (Brid.) Reim. (*Thuidium virginianum*).

Helodium blandowii (Web. & Mohr) Warnst.
Common in wet parts of bogs, swamps, and white spruce woods, shaded or otherwise, often associated with *Sphagnum* spp. *Polytrichum* spp., *Drepanocladus aduncus*, and *Aulacomnium palustre*.

Hylocomium splendens (Hedw.) B.S.G.
One of the common "feather mosses" of the region, variously associated with other mosses, or occasionally in nearly pure mats, on the floor of white spruce, poplar–spruce, and mature bog forests, also found on moist slopes under pine.

Hymenostylium recurvirostrum (Hedw.) Dix.
(*Gymnostomum recurvirostrum*)
M2086, M11061, on moist bank and tufa mound, iron springs, slope of wooded valley, White Mud Creek, Edmonton.

Hypnum cupressiforme L.
T9863, T10443, with *Abietinella* and other mosses, under poplar and white spruce, on clay bank of river, Fort Saskatchewan. M1958, damp parts of poplar stand, Villeneuve, 15 miles northwest of Edmonton.

H. patientiae Lindb.
(*H. lindbergii*)
Rather common in wet places, often with *Aulacomnium*, *Sphagnum* spp., *Climacium dendroides*, and *Ptilium crista-castrensis*.
T4340, wet ditch, in shade of poplar, Fort Saskatchewan. T10599, low place between *Sphagnum* hummocks, open bog, Pigeon Lake. M12337, moist shaded silt banks, near river, Edmonton.

H. vaucheri Lesq.
T9836, T9870, floor of white spruce woods, slope of river valley, near Fort Saskatchewan. Not clearly separable from *H. cupressiforme* and perhaps better treated as a variety of that species.

Leptobryum pyriforme (Hedw.) Wils.
Common on moist substrata of different kinds, often somewhat "weedy" on burned or otherwise denuded places, variously mixed with other mosses, in bogs, swamps, spruce stands, and mixed woods.

Leptodictyum riparium (Hedw.) Warnst. f. *longifolium* (Schultz) Moenk.
Usually in stagnant water and on wet mud after the water disappears. T1590 (sterile), in

deep pools, creek bed, T9868 (in fruit), on wet rocks in empty creek bed, both near Fort Saskatchewan. T10602a, on dead willows, just above the water, creek bank, Pigeon Lake.

L. riparium f. *trichopodium* (Schultz) Moenk.
(*L. trichopodium*)

T9762, on exposed root of a white spruce tree, associated with *Thuidium philibertii*, *Mnium* spp., *Climacium dendroides*, and *Rhodobryum roseum*, creek valley, Fort Saskatchewan. First report for Alberta.

L. kochii (B.S.G.) Warnst.
(*L. trichopodium* var. *kochii*)

T11286, with *Pylaisia*, *Mnium cuspidatum*, and *Brachythecium salebrosum*, on rotted wood, low poplar woods, near Fort Saskatchewan. First report for Alberta.

Leskea obscura Hedw.

T11232, on bare, rotted poplar log, low ground, under poplar trees, near Fort Saskatchewan. New to Alberta.

Meesia longiseta Hedw.

C. D. Bird 4312, wet edge of marl bog, 3 miles south of Heatherdown.

M. triquetra (L., Hook. & Tayl.) Aongstr.

M647, in a *Drepanocladus–Carex* bog, Nestow. M2762, open *Drepanocladus–Betula–Larix* bog, Peers.

M. uliginosa Hedw.

M9880, *Drepanocladus–Carex* bog, south of Hondo.

Mniobryum wahlenbergii (Web. & Mohr) Jenn.

Found usually in pure culture, on wet clay, face of creek or river bank, and submerged at highwater. T15, bank of Sturgeon River, northeast of Fort Saskatchewan. T1303, Whitemud Creek, Edmonton. T1606, bank of creek, and T10128, face of river bank, Fort Saskatchewan.

Mnium affine Funck var. *rugicum* (Laur.) B.S.G.
(*M. rugicum*)

Fairly common on low wet ground and banks, often beside pools, nearly always shaded, in various kinds of woodland and swamp. It thrives in wetter habitats than does the next species.

M. cuspidatum Hedw.

The most plentiful *Mnium* of the region, found in shaded habitats of various kinds, on a wide range of substrata—moist or dry ground, rotted wood, mossy banks and mounds, bases of trees. Associated mosses include *Pylaisia polyantha*, *Brachythecium salebrosum*, *Abietinella abietina*, and *Eurhynchium* spp.

M. drummondii Bruch & Schimp.

Edo Nyland 16, rotted birch stump, spruce forest, about 90 miles west of Edmonton.

M. medium B.S.G.

M10993, moist bank, poplar–spruce woods, Edmonton. T10557, damp ground in shade, black spruce bog, Elk Island Park, 20 miles east of Fort Saskatchewan. First report for Alberta.

Oncophorus wahlenbergii Brid.

M1965, in a large hollow charred log, near its open end, mixed woods, 20 miles east of Edmonton.

Orthotrichum elegans Hook. & Grev.

On dead, leaning willow trunks, 3 to 5 ft from the ground, on dead twigs of living spruce trees and on *Alnus tenuifolia* in low white spruce woods, on a rock in spruce woods, all at Fort Saskatchewan. On large trunks of *Populus balsamifera*, 2 to 7 ft from the ground, Edmonton and Villeneuve. On a dead spruce tree on the ground, low spruce woods, Battle Lake.

O. obtusifolium (Schrad.) Brid.

Common, 2 to 7 ft from the base, on sheltered trunks of large *Populus balsamifera*, rare on *P. tremuloides*, at Edmonton, Fort Saskatchewan, Elk Island Park, Villeneuve, and Gibbons,

20 miles north of Edmonton. Sometimes with *O. elegans* intermixed. Occasionally with *Pylaisia* spp. at the lower levels on poplar trunks.

O. striatum (L.) Hedw.
(*O. leiocarpum*)
Reported (10) for the region, under the latter name. This report was based on M1790, from Gibbons, consisting chiefly of *O. obtusifolium* but, as determined by A. L. Andrews, including some *O. leiocarpum*. Unfortunately, this species can not be found in the material now in hand.

Paludella squarrosa (Hedw.) Brid.
M8983, with *Sphagnum* and *Polytrichum*, *Picea–Larix* muskeg, Winterburn, near Edmonton.

Plagiothecium denticulatum (Hedw.) B.S.G.
M12344b, M12345, with *Brachythecium* spp., *Aulacomnium*, and other bryophytes, on ground and rotted wood, under alders and coarse grass, moat of *Sphagnum* bog, Boag Lake, near Edmonton.

Platygyrium repens (Brid.) B.S.G.
T9947, on rotted wood on ground, low white spruce woods, Pigeon Lake. M11075b, on rotted wood, *Larix–Picea* bog, 15 miles northwest of Edmonton.

Pleurozium schreberi (Brid.) Mitt.
(*Calliergonella schreberi*)
Widespread and often abundant in "feather moss' carpets of white spruce, climax black spruce, and other woodlands. Common associates are *Hylocomium*, *Ptilium*, *Aulacomnium*, and *Tomenthypnum*.

Pohlia camptotrachela (Ren. & Card.) Broth.
(*P. annotina*)
Reported as *Webera annotina* by Macoun (9) from springs, along Athabasca River, Fort Assiniboine.

P. cruda (Hedw.) Lindb.
T9957, with *Timmia megapolitana* and *Mnium cuspidatum*, under willows, slope of creek valley, Fort Saskatchewan.

P. nutans (Hedw.) Lindb.
Widespread, usually on decaying wood or peaty hummocks, rarely on damp soil, generally in deep shade, spruce and other woodlands.

Polytrichadelphus lyallii Mitt.
M8045, with *Sphagnum* and *Polytrichum*, open bog, near Lake Wabamun, west of Edmonton.

Polytrichum commune Hedw.
Rather common in *Sphagnum* bogs and tamarack swamps, occasional in alder–willow–poplar–spruce thickets.

P. juniperinum Hedw.
Very common, usually in bogs of various kinds and on rotted stumps or logs of spruce–poplar–birch woods, also in dry native grassland.

P. juniperinum var. *alpestre* B.S.G.
Bogs and open swamps. T23, bog in sand hills, north of Fort Saskatchewan. T11066, on *Sphagnum* hummock, cleared tamarack swamp, Pigeon Lake.

P. piliferum Hedw.
Common on dry ground of sand hills, either in the open or somewhat shaded by *Pinus* spp., occasional on bare areas in dry prairie grassland. Common associates are *Selaginella densa*, *Ceratodon purpureus*, *Peltigera*, and *Cladonia*.

Ptilium crista-castrensis (Hedw.) DeNot
(*Hypnum crista-castrensis*)
Occasional in white spruce and poplar–spruce forests, rather common in mature bog forests, as part of the "feather moss' stratum, the requisites being moist substratum and deep shade.

Pylaisia polyantha B.S.G.
Very common as a "stocking" around the bases of aspen and other trees, also on exposed roots of trees, variously mixed with other mosses. Other habitats are rotting baseboard of an old fence in shade of aspen, and cedar shingles on the north side of an old roof.

P. subdenticulata Schimp.
Not readily distinguished from the last species; may be rather common in the region. T10781, rotting log, deep poplar woods, Fort Saskatchewan. M11094a, well above the base, trunk of large balsam poplar, Edmonton. M12355, basal parts of trunks of white birch, moat of bog, Boag Lake, near Edmonton.

Rhodobryum roseum (Weis.) Limpr.
Associated with *Timmia megapolitana*, *Mnium cuspidatum*, and *Thuidium philibertii*. T10575 and T10577, under spruce, creek valley, near Fort Saskatchewan. First report for Alberta.

Rhynchostegiella compacta (C. Muell.) Loesk.
(*Amblystegium compactum*)
M11056, on old wood, moist bank, White Mud Creek, Edmonton.

R. compacta subsp. *americana* (Grout) Wijk. & Marg.
(*Amblystegium americanum*)
T10493, on ground under spruce, top of river bank, near Fort Saskatchewan. New to Alberta.

Rhytidiadelphus triquetrus (L., Hedw.) Warnst.
M2084, with *Pleurozium*, white spruce woods, Plamondon, 100 miles northeast of Edmonton. M1879, floor of poplar–spruce woods, Fort Assiniboine. M6917, boggy white spruce woods, White Mud Creek, Edmonton.

Rhytidium rugosum (Ehrh., Hedw.) Kindb.
T9749, T9774, under white spruce, on steep slope of river valley, near Fort Saskatchewan. M1478, top of butte, badland area, near Castor, about 125 miles southeast of Edmonton.

Scorpidium scorpioides (Hedw.) Limpr.
In water, usually with *Drepanocladus* spp. and other mosses, calcareous pools, marl bogs. M469, Calahoo. M11042, Acheson, near Edmonton.

Splachnum ampullaceum (L.) Hedw.
T920a, Fort Saskatchewan. M6831, muskeg, near McLeod River, north of Peers.

Tetraphis pellucida Hedw.
Associated with *Pohlia nutans*, *Mnium* spp., and *Dicranum* spp., often on rotted wood, also on old *Sphagnum* mounds and at bases of spruce trees, in woodlands and bogs.

Tetraplodon angustatus (Hedw.) B.S.G.
Reported by Macoun (9) from woods, between Edmonton and Lesser Slave Lake.

Thuidium philibertii Limpr.
T9716, wet ground, in shade of trees, near creek, Fort Saskatchewan. T9780, on very wet ground, heavy poplar cover, outlet of Battle Lake. All colonies associated with *Climacium dendroides*. This species seems to require wetter conditions than any other of our Thuidiaceae. First report for Alberta.

T. recognitum (Hedw.) Lindb.
Common in low white spruce woods, associated with *Pleurozium*, *Hylocomium*, and *Aulacomnium*, also in damp mixed woods and tamarack bogs.

Timmia bavarica Hessl.
M12332a, on moist rotting wood, floor of mixed woods, river valley, Edmonton.

T. megapolitana Hedw.
Numerous collections from shaded creek banks and damp floor of a valley, Fort Saskatchewan. Also from spruce–poplar woods, river valley, Edmonton.

Tomenthypnum nitens (Hedw.) Loesk.
(*Camptothecium nitens*)
Widespread, often abundant locally, in various kinds of low woods, boggy swamps, and open bogs.

Tortula mucronifolia Schwaegr.
Probably not uncommon, easily overlooked; collected from six stations, mostly moist and well shaded. T9678a, river bank, shaded by willows, Fort Saskatchewan. M1834a, damp ground, aspen grove, Viking, 75 miles southeast of Edmonton.

T. ruralis (Hedw.) Crom.
T9918, T10109, plentiful, mostly in pure cultures, on sand, under white spruce, along the water front, Ma-Me-O-Beach, Pigeon Lake.

Moss Communities

There are considerable bryological data in earlier papers (5, 10, 11) on the pine, poplar, and white spruce vegetation of the region. That information is augmented by certain floristic records and ecological notes in the first part of the present paper. Previous accounts (6, 7, 11, 12) of wet meadow, bog, and bog forest vegetation are also supplemented by data in the catalogue of this paper. However, some elaboration of the mosses of the latter vegetational types seems desirable, especially in terms of ecological relationships. There follows a brief treatment of the chief moss synusiae in several marshy and boggy associations.

Wet Meadow

In their pioneer work on marshes, wet meadows, and *Drepanocladus–Carex* bogs of central Alberta, Lewis *et al.* (7) show these to be of diverse types, though all are treated as "low moors". A marsh has been defined as a grass–sedge–rush community, usually without mosses or much peat accumulation and the floor covered with water during 1 or 2 months of the growing season. A shallow marsh, having water only a small part of the growing season, is a wet meadow. In our region, shallow marshes (wet meadows) are characterized by a thin moss stratum on the floor and there is some peat accumulation. Many of these meadows are gramineta, the leading grasses being *Calamagrostis canadensis*, *C. inexpensa*, *Glyceria* spp., and *Poa palustris*. Willow clumps are not uncommon. Indeed, the successional trend is to dominance by *Salix* spp. (7). A wet meadow may extend across a shallow depression or it may form a band around a pond or lake. Between the meadow and the water's edge there may be bands of wet marsh and reed swamp. Peripherally there usually are wooded zones dominated by *Salix* spp., *Alnus tenuifolia*, *Populus* spp., *Betula papyrifera*, and *Picea glauca*. Leading mosses in the thin stratum on the floor of the wet meadow are *Drepanocladus aduncus*, especially var. *kneiffii* and var. *polycarpus* f. *gracilescens*, also *Campylium radicale*.

Wet Thicket

This is the wooded vegetation associated with wet meadows and characterized by willows and coarse grasses. Accompanying plants include alder, balsam poplar, white birch, white spruce, sedges, many broad-leaved herbs, and bryophytes. Similar vegetation occurs in the peripheral depressions (moats) of many of the *Sphagnum* bogs of the region. Common mosses on the ground, or

on wet rotting wood close to the ground, are *Drepanocladus aduncus* and its varieties, *Aulacomnium palustre*, *Mnium affine* var. *rugicum*, *Climacium dendroides*, *Amblystegium juratzkanum*, and *Helodium blandowii*, while less-prevalent species are *Plagiothecium denticulatum*, *Campylium chrysophyllum*, and *Calliergon sarmentosum*. On drier mounds, also at tree bases, are *Drepanocladus uncinatus*, *Distichium capillaceum*, *Brachythecium salebrosum*, *Leptobryum pyriforme*, *Mnium cuspidatum*, *Pylaisia polyantha*, and many other mosses.

Drepanocladus–Carex Bog

Many of the undrained depressions of the region resemble marsh in general aspect but are characterized by a thick carpet of *Drepanocladus* moss and extensive peat formation, also by an abundant sedge growth and often a stratum of bog willows or bog birch (11). A depression may be almost completely occupied by a *Drepanocladus–Carex* bog or it may have a bog of this kind as a zone around a central lake. The chief mosses are *Drepanocladus aduncus*, *D. vernicosus*, and *D. revolvens* var. *intermedius*, these occurring in pure cultures or variously intermixed. They are sometimes largely submersed, but more often form a wet mat which is either resting on firm peat or held on a floating raft of vascular plants. The peat varies in thickness from a few decimeters to many meters. As reported in earlier papers (6, 7) the pH of these bogs ranges from 5.5 to 6.5.

Mosses commonly associated with *Drepanocladus* spp. include species of *Meesia*. The vascular flora varies considerably from bog to bog, depending on depth of water and on obscure factors. Leading sedges are *Carex diandra*, *C. lasiocarpa* var. *americana*, and *C. aquatilis*. The chief shrubs are the bog willows, *Salix pedicellaris* var. *hypoglauca* and *S. candida*, and the bog birch, *Betula pumila* var. *glandulifera*. Associated plants include *Eriophorum* spp., *Carex* spp., *Muhlenbergia glomerata* var. *cinnoides*, *Agrostis scabra*, *Equisetum fluviatile*, *Potentilla palustris*, *Menyanthes trifoliata*, *Aster junciformis*, *Triglochin maritima*, and *Sium cicutaefolium*. The roots and rhizomes of several of these vascular species comprise a supporting raftlike structure for *Drepanocladus* growth over liquid peat in central areas of bogs and at the invading edges of bog lakes.

Sphagnum Bogs

These bogs have developed either from *Drepanocladus–Carex* bogs or from aquatic-marsh vegetation without the interpolation of marked *Drepanocladus* phases (7, 11). The ecological relationships of various species of *Sphagnum* in Alberta bogs have been discussed in earlier papers (11, 12). For the Edmonton region, succession from initial aquatic to climax stages may be shown as follows: *Sphagnum subsecundum* (or, *S. teres*) → *S. recurvum* → *S. magellanicum* (or, *S. warnstorfii*) → *S. capillaceum* (or *S. capillaceum* var. *tenellum*) → *S. fuscum*. This successional series is observable in wet depressions of bogs, also where *Sphagnum* is advancing upon marsh or *Drepanocladus–Carex* bog.

The "regeneration complex", discussed for Alberta bogs by Marion Moss (12), is the condition of a developing bog where the majority of the hummocks are covered by the more xerophilous species of the *Sphagnum* sere, and where the hollows are occupied by relatively hygrophilous species. "The aspect is a

mosaic of hummocks and hollows occupied by different species, and the development is cyclic, the hollows becoming the sites of mounds, and the mounds the sites of hollows, through a series of *Sphagnum* sequences. The state of dynamic equilibrium inherent in the cycle is followed, sooner or later, by a condition of static equilibrium, viz. when the general surface of the bog has become too dry for any of the sphagnums except *S. fuscum*" (11).

Bryophytes commonly associated with *Sphagnum* spp. in the bogs include *Polytrichum* spp., *Aulacomnium palustre*, *Tomenthypnum nitens*, *Dicranum bergeri*, and the leafy liverwort, *Mylia anomala*.

Open *Sphagnum* bogs tend to develop into bog forest. Retrogression to open bog and to earlier stages of the bog sere may occur through burning. Repeated surface-burning of a relatively mature bog or bog forest tends to produce a somewhat static bog phase characterized by low hummocks of *Sphagnum fuscum*, scattered black spruce, tamarack, white birch, and willows, a dense growth of *Ledum groenlandicum*, tussocks of *Eriophorum vaginatum* subsp. *spissum*, and varying amounts of *Vaccinium* spp., *Cladonia* spp., *Polytrichum commune*, *P. juniperinum*, and other mosses.

Bog Forests

These forests are of three main types: (*a*) black spruce – bog moss (*Picea mariana–Sphagnum*) association, being an advanced stage of the *Sphagnum* bog sere; (*b*) tamarack (*Larix laricina*) association, an advanced stage of a *Drepanocladus–Carex–Betula* bog, usually with extensive *Sphagnum* mounds; (*c*) black spruce – feather moss association, generally regarded as a climax bog forest developed from either of the preceding associations. Since these communities have been considered at some length in earlier papers (6, 7, 11), the following treatment is confined to an elaboration of the moss synusiae.

Black spruce – bog moss association.—The floor is characterized by: (*a*) bog mosses, chiefly *Sphagnum capillaceum*, *S. fuscum*, *S. magellanicum*, *S. warnstorfii*, *S. recurvum*, *Aulacomnium palustre*, and *Tomenthypnum nitens*; (*b*) the "*feather mosses*" *Pleurozium schreberi*, *Hylocomium splendens*, *Ptilium crista-castrensis*, and *Thuidium recognitum*, these mostly confined to the higher mounds and decayed wood. Occupying a variety of "niches" are the secondary moss species *Helodium blandowii*, *Polytrichum* spp., *Mnium* spp., *Eurynchium pulchellum*, *Pohlia nutans*, *Leptobryum pyriforme*, *Haplocladium microphyllum*, *Amblystegium serpens*, *Brachythecium salebrosum*, *Bryum* spp., *Dicranum* spp., *Tetraphis pellucida*, *Campylium chrysophyllum*, *Bryoerythrophyllum recurvirostre*, *Drepanocladus uncinatus*, *Pylaisia* spp., also the leafy liverworts *Lophozia incisa*, *L. porphyroleuca*, *Riccardia latifrons*, *Blepharostoma trichophyllum*, and many other species.

Tamarack association.—This community presents many facies, depending on water level, age, and density of the trees, extent of burning, sawfly depredation, and other factors. In general, the bryophytic flora is that of the last association. Additional mosses of some significance are *Brachythecium acutum* (often in mats on the floor, on rotting wood, and at bases of small tamarack trees), *Drepanocladus aduncus*, and *Calliergon cordifolium* (in pools between mounds, in wet facies of the association).

Black spruce – feather moss association.—The bryophytes of this com-

munity are essentially the dominant "feather mosses", along with most of the secondary species, listed for the black spruce – bog moss association. The gradual transition from one association to the other is marked by an elevation of the floor (through accumulation of moss peat and partially rotted wood), and a concomitant replacement of *Sphagnum* by feather mosses and other species. This transition provides a good example of a bryophytic "continuum".

Marl Bogs

Several of these bogs are known for the Edmonton region, most of them 5 to 40 miles west and northwest of the city. They are usually associated with *Sphagnum* bogs and bog forests, rarely with spruce–poplar woods on flats below a valley slope. Most of them are in the form of islands or strips extending through extensive bog forests. The bog surface consists of peaty mounds, mossy mats, marl flats, and shallow pools of alkaline water, the bottoms of the pools being whitish with calcareous deposit. The ponds and small lakes of certain of these bogs described in earlier papers (6, 7) have virtually disappeared through drainage of the areas. Chemical analyses showed these ponds to be very rich in mineral salts, chiefly calcium carbonate. Apparently, lime-charged water from springs seeps in slowly to produce conditions suitable for this unusual kind of bog development and a characteristic flora. There is good evidence of bog forest having been converted to marl bog in the past (6, 7). However, certain of the bog forest and marl bog areas, known to the senior author for some 35 years and not extensively drained or otherwise disturbed during this period, seem to be in a state of equilibrium, or nearly so. Brief accounts of the vegetation of our marl bogs have been published (6, 7, 14). These descriptions will be co-ordinated and elaborated here, with particular attention to the mosses.

The larger mounds of a typical marl bog support a mixed flora of small trees, shrubs, herbs, and mosses akin to that of *Sphagnum* bogs, viz. black spruce, tamarack, white birch, bog birch, Labrador tea, *Vaccinium vitis-idaea* var. *minus*, *Oxycoccus microcarpus*, *Andromeda polifolia*, *Rubus acaulis*, *Carex* spp., *Tomenthypnum nitens*, *Polytrichum* spp., *Sphagnum* spp., (often *S. capillaceum* var. *tenellum*), and "feather mosses", especially *Pleurozium schreberi*.

The lower peaty mounds, the extensive wet mossy mats, the marl flats, pool margins, and the pools themselves are tenanted by the characteristic marl bog flora. Leading mosses of low mounds are *Tomenthypnum nitens* and *Aulacomnium palustre*, and of wet mats, *Campylium stellatum*. Other mosses recorded for low mounds and wet mats are *Distichium capillaceum*, *Catoscopium nigritum*, and *Bryoerythrophyllum recurvirostre*. Common vascular plants of low mounds, mossy mats, and marl flats are *Gentianella crinata* subsp. *macounii*, *Pinguicula vulgaris*, *Scirpus caespitosus* var. *callosus*, *S. pumilus* subsp. *rollandii*, *Eleocharis pauciflora* var. *fernaldii*, *Carex viridula*, *Juncus* spp., *Lobelia kalmii*, *Triglochin* spp., *Allium schoenoprasum* var. *sibiricum*, *Tofieldia* spp., *Dodecatheon radicatum*, and *Selaginella selaginoides*.

The pools and ponds are usually tenanted by *Scorpidium scorpioides* and *Drepanocladus* spp., one or more of *D. revolvens*, *D. revolvens* var. *intermedius*, and *D. aduncus*, often with *Campylium stellatum* at the edges. Associates in

this synusia are *Bryum pseudotriquetrum*, *B. pallens*, *Meesia longiseta*, and *Amblyodon dealbatus*. Other plants of the calcareous pools are *Carex livida*, *Utricularia* spp., *Chara* sp., and algae—green, blue green, and diatoms. For lakes and ponds of marl bogs, Lewis and Dowding (6) record numerous algal species, some present in great abundance. The algae and *Chara* are considered to be "the chief agents responsible for the great precipitation of mineral salts above the floor of the lake". Our observations indicate that, at least in the shallow ponds and pools and in the mossy mats, the aquatic and semiaquatic mosses also have some part in the precipitation and deposition of calcareous material.

In terms of leading mosses, the main hydrarch sere for marl – peat moss bogs may be set forth as follows: *Scorpidium scorpioides*+*Drepanocladus* spp. → *Campylium stellatum* → *Aulacomnium palustre*+*Tomenthypnum nitens* → *Sphagnum* spp. → *Pleurozium*+*Hylocomium*.

References

1. Andrews, A. L. 1951. Studies in the Warnstorf Sphagnum Herbarium VI. The subgenus Inophloea in the Eastern Hemisphere. Bryologist, **54**, 83–91.
2. Bird, C. D. 1960. A preliminary annotated catalogue of the bryophyte flora of Alberta. (Mimeographed.) Botany Department, University of Alberta.
3. Brinkman, A. H. 1923. List of Alberta hepatics. Can. Field-Naturalist, **37**, 105–106.
4. Brinkman, A. H. 1929. Hepatics and sites: a short study in the ecology of hepatics. Bryologist, **32**, 29–30.
5. Dowding, E. S. 1929. The vegetation of Alberta. III. The sandhill areas of central Alberta with particular reference to the ecology of *Arceuthobium americanum* Nutt. J. Ecol. **17**, 82–105.
6. Lewis, F. J. and Dowding, E. S. 1926. The vegetation and retrogressive changes of peat areas ("muskegs") in central Alberta. J. Ecol. **14**, 317–341.
7. Lewis, F. J., Dowding, E. S., and Moss, E. H. 1928. The vegetation of Alberta. II. The swamp, moor and bog forest vegetation of central Alberta. J. Ecol. **16**, 19–70.
8. Macoun, J. 1890. Contributions to Canadian bryology. No. 2. Bull. Torrey Botan. Club, **17**, 83–90.
9. Macoun, J. 1892. Catalogue of Canadian plants. VI. Musci. Montreal.
10. Moss, E. H. 1932. The vegetation of Alberta. IV. The poplar association and related vegetation of central Alberta. J. Ecol. **20**, 380–415.
11. Moss, E. H. 1955. The vegetation of Alberta. Botan. Rev. **21**, 493–567.
12. Moss, M. S. 1949. Taxonomic and ecological studies of *Sphagnum* species and bogs in Alberta. Unpublished Thesis, Univ. Western Ontario, London.
13. Steere, W. C. 1950. On the distribution of *Aloina brevirostris* in North America. Bull. Torrey Botan. Club, **77**, 503–508.
14. Turner, G. H. 1949. Plants of the Edmonton district of the Province of Alberta. Can. Field-Naturalist, **63**, 1–28.
15. Welch, W. H. 1960. A monograph of the Fontinalaceae. Maritinus Nijhoff. The Hague.
16. Wynne, F. E. 1944. Studies in Drepanocladus. IV. Taxonomy. Bryologist, **47**, 147–189.

A FUSARIUM CANKER DISEASE OF POPULUS DELTOIDES MARSH.[1]

M. G. BOYER[2]

Abstract

Two morphologically distinct types of *Fusarium solani* sensu S. & H. induce a characteristic canker disease on *P. deltoides* on low-lying sites in Quebec. Studies on the morphology and pathogenicity of the causal organism are presented.

Introduction

Following a survey of diseases of native and introduced poplars in Quebec a more intensive program of study on the biology of specific pathogens was initiated. This paper concerns primary investigations and observations made on a canker disease of *Populus deltoides* caused by *Fusarium solani* sensu S. & H.[3]

Species of the genus *Fusarium* have at various times been reported to be pathogenic on poplar. In Canada, *F. lateritium* Nees has been identified as the cause of a canker disease on *P. trichocarpa* Torr. & Gray (3), while in Europe *F. avenaceum* (Fr.) Sacc. was found to be pathogenic on *P.* × *euramericana* cv. robusta (16). *F. bulbigenum* Cke. & Mass. has also been reported as pathogenic on poplar, though under what conditions is not stated (4). Other references to *Fusarium* spp. on poplar even as saprophytes are relatively rare in check lists of the fungi. Seymour (14) lists *F. radicicola* Wr. on *P. deltoides* apparently a parasite, while *F. sporotrichioides* Sherb and *F. acuminatum* Ell. & Ev. have been found on dead branches and decaying wood of *P. tremuloides* (8). Mention is also made of species of *Fusarium* occurring as contaminants in isolations made from cankers on hybrid poplars caused by *Septoria musiva* Pk. (18). However, it is rather interesting that in spite of the wide geographical distribution and host range of this genus many studies and references to the fungal flora of poplar in North America (1, 6, 15) do not list the occurrence of *Fusarium*.

Fusarium solani is one of the more ubiquitous saprophytes of the genus (4, 7, 8). As with most species of *Fusarium* it is apparent that diverse forms of the species exist, varying in pathogenicity. Thus, the organism has been found to be pathogenic on various hosts, such as potato (5), citrus fruits (4), clover (9), and seedlings of conifers (12), generally being associated with storage or root rots. It has also been reported as the cause of blue stain in woody plants (11), but apparently has not previously been found as the causal agent of a canker disease.

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Contribution No. 655 from the Forest Biology Division, Research Branch, Department of Agriculture, Ottawa, Canada.
[2]Forest Biology Laboratory, Quebec, Que.
[3]All the cultures of *Fusarium* were kindly identified by Dr. W. L. Gordon, Canada Department of Agriculture, Winnipeg, Manitoba.

Methods

In a stand near Berthierville, Quebec (Figs. 1 and 2), an area 100 feet square containing 183 trees was established to study the canker disease. The trees were numbered and the d.b.h.,[4] crown density, and sex, where possible, recorded for individual trees. Increment borings were taken to obtain tree and canker ages. The average annual radial increment, called here the growth index, was determined for each tree. These measurements were then arranged in growth index classes for the purpose of comparing growth with the incidence of the disease.

Isolations from representative cankers were made in the laboratory by plating aseptically cut chips from the advancing margin of the lesions. Dead branches, green shoots, and the subcortical tissue surrounding the base of shoots of adventitious or axillary origin were also cultured. In the latter case isolations were made successfully in the field by sterilizing the bark with full-strength sodium hypochlorite (Javex), shearing the bark off aseptically, and plating a sample from the living tissue surrounding the shoot origin. Suspected organisms isolated in this manner were inoculated on growing cuttings of *P. deltoides* in the greenhouse. A uniform piece of the bark tissue was removed with a knife and a block of agar containing the fungus and of approximately the same size placed firmly against the wound. The stem above and below was then wrapped with a small piece of moist absorbent cotton and the whole covered with aluminum foil and tied. The foil was removed after 1 month and at 3 months the cankers were measured and reisolation attempted from samples of each suspected pathogen. In addition to triplicate inoculations of the predominant isolates, duplicate inoculations were carried out with other suspected organisms isolated during this study. Wounded cuttings which were not inoculated were used as controls (Fig. 13).

General Site Characteristics

Although *P. deltoides* and *P. eugenii* Simon-Louis have been extensively planted throughout most of the settled areas of Quebec, the native species reaches its northernmost natural extent in this province along the St. Lawrence River near Trois-Rivières. In this area it occurs sporadically on river banks and other moist low-lying sites, either in pure stands or in association with *Salix* spp.

The observations and investigations reported here were made on the low-lying alluvial flats extending out into the St. Lawrence from Berthierville, 40 miles northeast of Montreal, towards the south shore of the river. The general characteristics of the site under study appeared to be uniform and typical of stands in the area, although a detailed survey was not attempted. The topography is uniformly flat. The soil is predominantly a gray sand and silt mixture of slightly acid pH overlying a neutral clay and silt subsoil. Water table measurements varied from 2 to 3 feet in June 1959 to about 5 feet in the late summer although, during some years, flooding of the area occurs[5] in early spring. While the light-textured soils of neutral pH observed here are similar

[4]d.b.h. = diameter at breast height.
[5]Dr. R. Pomerleau, personal communication.

PLATE I

FIG. 1. A general view of the stand of *P. deltoides* in which the plot was located.
FIG. 2. The interior of the stand.
FIG. 3. A shoot arising from adventitious bud development naturally infected with *F. solani* type (*a*).
FIG. 4. A shoot infected by the three types of *F. solani*.
FIG. 5. A cluster of shoots on which *Fusarium* spp. were fruiting.
FIG. 6. *F. solani* type (*a*) growing from the extracted pith of a living shoot.
FIG. 7. A 6-year-old canker on a tree 7 years old.
FIG. 8. A 3-year-old canker on a tree 6 years old.
FIG. 9. Typical cankers from which *F. solani* was isolated. The bark was removed from the canker at left.

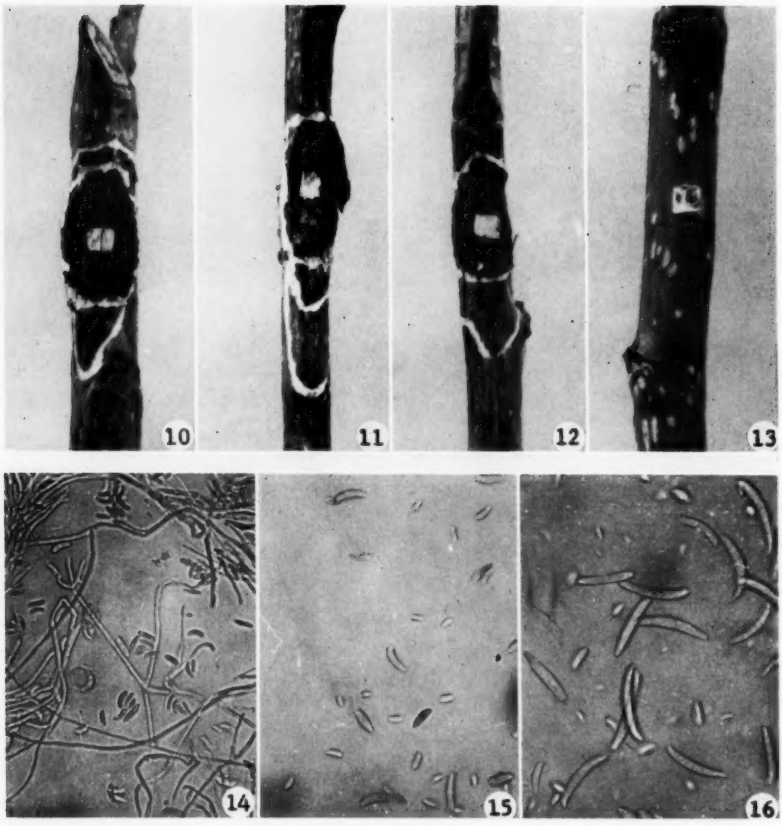

FIG. 10. A canker resulting from inoculation by *F. solani* type (*a*) after 3 months on a cutting of *P. deltoides*. The point of inoculation is indicated in white.
FIG. 11. A canker resulting from inoculation by *F. solani* type (*a*) after 3 months. The lines indicate extent of the canker from where the organism was reisolated.
FIG. 12. A canker resulting from inoculation by *F. solani* type (*b*) after 3 months.
FIG. 13. Control.
FIG. 14. Spores of *F. solani* type (*a*).
FIG. 15. Spores of *F. solani* type (*b*).
FIG. 16. Spores of *F. solani* type (*c*).

to those found associated with superior poplar sites (13), shallow rooting and the presence of *Phalaris arundinacea* L. as the primary component of the ground cover would suggest that drainage is a detrimental factor on this site (13).

Characteristics of the Disease

In the plot, 84 trees, or 46 per cent, had one or more cankers varying from large basal cankers 30 to 40 cm in length to small lesions 1 to 2 cm in length (Fig. 9). Typically, cankers from 2 to 5 years old are much elongated with very irregular margins. Retention of the bark in patches, where the cambium is not entirely destroyed, gives a rough, uneven appearance to the canker. Distortion of the stem frequently resulted from the often sporadic development of the causal agent and the excessive amount of callus formed. Occasionally, actively growing cankers from which the causal agent was isolated were found under the bark of older trees (Fig. 9). Girdling was not observed and consequently trees were never killed directly by the canker but were often so weakened they succumbed to wind throw or other fungi, such as *Valsa nivea* (Hoff.) Fr. and *V. sordida* Nit. Fructifications of these fungi and *Fusarium* spp. were observed on the margins of older cankers, and often in the case of *Valsa* they extended beyond the extremities of the lesions.

Age determinations were made on 30 typical cankers in the stand. Infection of 27 of them, or 90 per cent, took place when the stem at the point of origin of the canker was from 1 to 3 years old; that is, prior to active phellogen development (Figs. 7 and 8). The remaining cankers were observed on older trees and found to have occurred when the trees were 4 or more years of age.

The relationship between age and growth, sex, and the incidence of the disease was investigated. Age variation within the plot was from 5 to 18 years. The sample size in some age groups was not large, but there was no apparent tendency for the disease to be confined to any particular age group (Table I).

TABLE I

The distribution of *Fusarium* canker with age of trees

Age of trees	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Number of trees	2	3	13	35	36	33	18	11	10	8	5	4	4	1
Number infected	1	3	9	17	10	16	8	5	6	3	3	3	0	0

Thirteen trees in the plot were identified as females and 97 as males and, of these, 5 females and 32 males were diseased. This sample was insufficiently large to make a quantitative comparison. However, a slight preponderance of diseased female trees suggests that a possible relationship exists between sex and disease resistance. This has been indicated in other species of poplar (10).

A distinct inverse trend existed between the growth index and the incidence of the disease (Fig. 17). Since this numerical estimate of growth showed a close relationship to the visual classification of dominance or suppression based on crown classes, it is probable that suppressed trees are more susceptible to infection. It is difficult to evaluate what effects, if any, the canker has in this relationship. An attempt was made to correlate the growth index with the number of cankers per tree on the assumption that if the canker has a measur-

able effect on growth, trees with several cankers would exhibit a lower growth index than trees with one. However, no such relationship was evident.

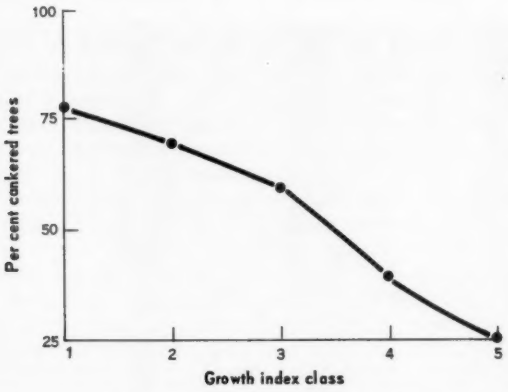

FIG. 17. The relationship between the growth index class and the per cent of cankered trees. Values of the growth index for each growth index class are: 1 = 0.05–0.08, 2 = 0.09–0.12, 3 = 0.13–0.16, 4 = 0.17–0.20, 5 = above 0.20.

Isolations of the causal agent of this disease were attempted from the entire circumference of 18 actively growing and 5 partially healed cankers. From actively growing lesions 1 to 4 years old, a series of *Fusarium* isolates were obtained. These organisms were often found growing together on the same canker but a mixture of two or more could generally be separated when relatively large numbers of isolations from each canker had been made and the fungi selected by hyphal-tip transfer. Once obtained, the cultures were subsequently checked for purity by single-spore isolation. All four types showed distinctive and constant cultural characteristics. Only rarely were other organisms isolated from the cankers. The results from these studies are summarized in Table II with the *Fusaria* arranged in order of prevalence. Occasional contamination by known saprophytes was not included.

Of the four *Fusaria*, only one, *F. solani* type (*a*), was isolated in the absence of the others in 5 of the 18 cankers. In addition this isolate was the most prevalent. *Valsa sordida* and *V. nivea* were commonly isolated in association with the cankers but appeared to be ubiquitous and often sporulated profusely far beyond the visible margins of the canker on apparently normal bark. *Cryptodiaporthe populea* (Sacc.) Butin was obtained from one canker in association with three of the four *Fusaria*.

From the five partially healed cankers the *Fusaria* were isolated twice. *Valsa* was common and both species were isolated from four of the five cankers. *C. populea* was not observed.

On soft green shoots arising as a result of axillary or adventitious bud development (Fig. 5) lesions were often observed from which *F. solani* was consistently isolated. Petiolar infection was common (Figs. 3 and 4), penetrating

TABLE II

Frequency of isolation of fungi from cankers on *P. deltoides*

Isolates	Canker No.																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Fusarium solani (*a*)	+		+	+	+	+	+		+		+		+		+	+	+	+
Fusarium solani (*b*)		+	+		+	+	+	+		+	+	+		+				
Fusarium solani (*c*)		+			+		+	+		+		+	+	+				
Fusarium avenaceum		+				+	+			+		+						
Valsa nivea						+						+	+		+			
Valsa sordida			+				+					+				+		
Cryptodiaporthe populea					+													

rapidly to the main shoot. These green shoots, which rarely survive more than 2 years, have soft and poorly lignified woody tissue and a large and spongy pith. Infection generally remained localized until the death of the shoot, when rapid colonization took place. The pathogen progressed through the xylem to the pith and eventually to the base of the shoot. Isolations made from the cortical and pith tissue of dying shoots yielded abundant colonies of *Fusarium* (Fig. 6). The subcortical tissue surrounding the shoot origin was also colonized by the pathogen when it had initially succeeded in penetrating to the base of the shoot internally. This was indicated by isolations made concurrently from the pith of the shoots and the tissue surrounding the shoot origin by the methods previously given. Cankers thought to have arisen from this source are illustrated in Fig. 9. Dead branch stubs, 3 to 4 years old when cultured, frequently yielded colonies of *Fusarium* and the possibility that these also serve as a source of infection seems feasible. However, no cankers typical of the disease could be traced to this origin.

All the organisms isolated from the cankers were tested for pathogenicity on rooted cuttings of *P. deltoides*. In addition, two other organisms prevalent on this host and known to induce cankers on poplar were included in the test. *Mycosphaerella populorum* Thompson was a common foliage pathogen in the plot that had been shown previously to induce cankers on hybrid poplars (18), while *Myxosporium* sp. was observed in association with a dieback on *P. deltoides* and is identical with the imperfect stage of *Neofabrea populi* Thomp., a pathogen on *P. tremuloides* (17). The data from the experiment are summarized in Table III.

TABLE III

Pathogenicity of fungi isolated in the plot

Organism	No. of successful inoculations	Average extent of canker (cm)	Reisolation
Fusarium solani (*a*)	3	4.5	+
Fusarium solani (*b*)	3	4.5	+
Fusarium solani (*c*)	2	1.5	+
Fusarium avenaceum	1	0.5	+
Valsa nivea	1	2.0	+
Valsa sordida	2	2.0	+
Cryptodiaporthe populea	2	2.5	+
Mycosphaerella populorum	0	0.5	−
Myxosporium sp.	0	0.5	−
Control	—	0.5	−

Types (*a*) and (*b*) of *F. solani* were pathogenic on the cuttings (Figs. 10, 11, and 12) and were successfully reisolated from the advancing margin of the lesions both at 3 months and at 5 months after inoculation. *F. solani* (*c*) appeared to be slightly pathogenic, but was not reisolated at 5 months. *F. avenaceum* was nonpathogenic, although it was reisolated from one cutting 3 months after inoculation. The possibility that these organisms together might exert a more pathogenic effect was not investigated at this time. The development of the cankers induced by the known pathogens, *V. nivea*, *V. sordida*, *C. populea*, appeared to be slower, but they were nevertheless successful.

TABLE IV

Spore measurements and growth rates of the three isolates of *F. solani*

Isolates	Macrospore size (μ)		Septae	Microspore size (μ)		Pigmentation	Radial growth* (mm)
	Length	Width		Length	Width		
F. solani (*a*)				5.7 (3.6–12.0)	3.0 (2.0–4.5)	Faint pink	44
F. solani (*b*)	18.0 (12.0–24.6) (Very sparse)	3.6 (2.4–4.8)	1(0–3)	5.9 (2.4–10.8)	2.8 (2.4–4.8)		39
F. solani (*c*)	36.2 (24.0–49.2)	5.5 (3.6–7.2)	3(0–5)	5.6 (3.6–12.0)	3.0 (2.4–4.8)	Pink	53
	24.0–24.4	3.6–6.0	0				
	31.2–49.2	4.8–7.2	3				

*Average of four cultures grown at 22° C on Difco P.D.A. after 8 days.

Evidence that these three organisms are pathogenic on trees weakened by drought or other environmental factors has been presented before (19, 20). Neither *M. populorum* nor *Myxosporium* sp. was reisolated, and they appeared to be nonpathogenic under these conditions. Evidence that *P. deltoides* was resistant to *M. populorum* has been presented previously by Bier (2).

Morphology of Isolates

The three types of *Fusarium solani* isolated from actively growing cankers were studied *in vitro*. Morphological observations served to differentiate them (Table IV and Figs. 14, 15, and 16). Measurements were taken on 100 spores mounted in lactophenol and taken from cultures grown on Difco P.D.A. 20 days after isolation at 22° C.

Gross morphological characteristics of cultures grown on P.D.A. in diffuse light are briefly described below:

Fusarium solani type (*a*).—Mycelium at first white, fluffy, turning fawn-colored with age. Older colonies forming a loose surface mat appressed to the agar, and covered with a dense powdery mass of spores which often collapse giving the cultures a ragged water-soaked appearance. Agar color at first pink, later turning brown.

Fusarium solani type (*b*).—Mycelium white to cream-colored, sparse, always closely appressed to agar. Whole colony early becoming yeastlike with dense zonate masses of spores. No pigmentation in agar.

Fusarium solani type (*c*).—Colonies initially white, later turning dark brown. Central part of colony only becoming moist and yeastlike. Agar color at first pink, later turning red.

Discussion

As a result of preliminary studies on a canker disease of *P. deltoides*, pathogenic forms of *Fusarium solani* have been isolated and shown to be both constantly associated with the disease and pathogenic on wounded cuttings of the host. Since a weakly pathogenic form of the species was also isolated, it appears probable that the organism consists of several forms differing in morphological and pathological characteristics.

The association of these forms and of *F. avenaceum*, so frequently found when isolations were made from the cankers, suggests that together an enhancement of pathogenicity might result. However, two of them were found to be non-pathogenic or only weakly pathogenic when inoculated singly.

Infection appears to arise primarily on young stems 1 or 2 years old, the fungus remaining active for periods up to 5 years. Only rarely did cankers originate on older parts of the trees, apparently because of the development of a thick bark after the third year. The organism gains entrance through wounds or through dead or dying shoots arising from adventitious bud development. In the latter case the pathogen moves through the pith of the shoots into the subcortical tissue of the stem.

The prevalence of *Valsa nivea* and *V. sordida* and their widespread distribution in the area suggests that they might also play a role in the development of the disease. This may account for the large size attained by some of the older cankers. While *Cryptodiaporthe populea* was isolated only once from the can-

kers, it is possible that this fungus is involved to some extent perhaps in the early stages of development.

Though comparative stands of equal age growing on different sites were not studied, this disease has not been identified on the native cottonwood or on related poplars other than in this area. At least two factors appear to be important in the localization and severity of the disease. Since an inverse relationship existed between the growth index and the incidence of the disease, suppression through overstocking would be a natural consequence where site conditions are conducive to rapid growth. While appropriate stand density tables are not available, the fact that 106 of the 190 trees have a growth index less than the average of the stand (0.18) would suggest that overstocking exists with the resultant suppression of many of the trees.

Probably of equal importance in the establishment of the disease are microclimatological factors. Both *Fusarium solani* (*a*) and (*c*) sporulate abundantly on dead branches and trunks as well as at the margins of some cankers under the conditions existing on this site, providing sufficient inoculum for widespread infection.

Sound cultural practices would probably eliminate the disease. Where stands of *P. deltoides* have developed as a result of wind- or water-borne seed, overstocking in the early years is probably a common occurrence. Thinning of the stand through removal of suppressed or weakened trees, where this is economically sound, would not only reduce the incidence of the disease but also greatly enhance the productivity of these potentially high-yielding sites.

Acknowledgments

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References

1. BASHAM, J. T. 1958. Decay of trembling aspen. Can. J. Botany, **36**, 491–505.
2. BIER, J. E. 1939. *Septoria* canker of introduced and native hybrid poplars. Can. J. Research, C, **17**, 195–204.
3. BIER, J. E. 1959. The relation of bark moisture to the development of canker diseases caused by native facultative parasites. 2. *Fusarium* canker on black cottonwood. Can. J. Botany, **37**, 781–788.
4. BOURGIN, G. V. 1949. Les champignons parasites des plantes cultivées. Tome II. Masson et Cie, Paris. pp. 1567–1569.
5. DYKSTRA, T. P. 1941. Potato diseases and their control. U.S. Dept. Agr. Farmers' Bull. 1881. p. 23.
6. ETHERIDGE, D. E. and LAUT, J. 1958. Fungi associated with living and dead branches of pole-size aspen. Bi-Monthly Progr. Rept., Div. Forest Biol., Can. Dept. Agr. **14** (4), 2–3.
7. GORDON, W. L. 1952. The occurrence of *Fusarium* species in Canada. II. Prevalence and taxonomy of *Fusarium* species in cereal seed. Can. J. Botany, **30**, 209–251.
8. GORDON, W. L. 1959. The occurrence of *Fusarium* species in Canada. VI. Taxonomy and geographic distribution of *Fusarium* species on plants, insects, and fungi. Can. J. Botany, **37**, 257–290.
9. KILPATRICK, R. A. and HANSON, E. W. 1950. Root and crown rots of red clover in Wisconsin. Phytopathology, **40**, 16.
10. PAULEY, S. S. 1949. Forest-tree genetics research: *Populus* L. Econ. Botany, **3**, 299–330.
11. SCHEFFER, T. C. and LINDGREN, R. M. 1932. Some minor stains of southern pine and hardwood lumber and logs. J. Agr. Research, **45**, 233–237.
12. POMERLEAU, R. 1932 and 1934. The fungi responsible for seedling blight of conifers at the Berthierville forest nursery. Que. Soc. Prot. Plants, 25th and 26th Repts. pp. 59–62.

13. SCHREINER, E. J. 1959. Production of poplar timber in Europe and the significance and application in the United States. U.S. Dept. Agr. Forest Serv. Agr. Handbook No. 150. pp. 38 and 42.
14. SEYMOUR, A. B. 1929. Host index of the fungi of North America. Harvard Univ. Press, Cambridge, Mass., U.S.A. p. 190.
15. SHAW, C. G. 1958. Host fungus index for the Pacific Northwest. Vol. II. Fungi. Wash. Agr. Expt. Sta. Circ. 335.
16. TARIS, B. 1957. Contribution à l'étude des maladies cryptogamiques des rameaux et des jeunes plants de peuplier. Maison Poulet-Malassis, Alencon (Orne), France.
17. THOMPSON, G. E. 1939. A canker disease of poplars caused by a new species of *Neofabraea*. Mycologia, **31**, 455–465.
18. WATERMAN, A. M. 1954. *Septoria* canker of poplars in the United States. U.S. Dept. Agr. Circ. 947.
19. WATERMAN, A. M. 1957. Canker and dieback of poplars caused by *Dothichiza populea*. Forest Sci. **3**, 175–183.
20. WRIGHT, E. 1957. Cytospora canker of cottonwood. U.S. Dept. Agr. Plant Disease Reptr. **41**, 892–893.

MARINE NANNOPLANKTON FROM A BRITISH COLUMBIA FJORD[1]

ROBERT F. SCAGEL AND JANET R. STEIN

Abstract

The qualitative aspects of the nannoplankton in Indian Arm, British Columbia, have been heretofore unknown, although the relative quantitative significance of this difficult group of unicellular organisms is apparent from recent primary production studies using the carbon-14 method. This qualitative study of samples collected in the spring of 1960 in Indian Arm has added a number of new records of distribution for the area (*Chromulina* sp., *Ochromonas ?vallesiaca* Chodat, *Chrysochromulina* sp., *Thallasomonas exurgens* Butcher); and has led to the discovery of several new species and genera (*Chrysamoeba nana* sp. nov., *Microsportella* gen. nov. *fiordensis* sp. nov., *Xanthomonas* gen. nov. *thalassoides* sp. nov., *Pseudomicrosportella* gen. nov. *ornata* sp. nov., and *Anisomonas astigmatica* sp. nov.). The study is being continued to determine qualitatively the seasonal and three-dimensional distribution of these and other nannoplankton in the fjord in relation to the physical-chemical aspects of the environment.

Introduction

As a result of recent primary production studies using the carbon-14 method in Indian Arm, British Columbia, it has become apparent that the nannoplankton play a significant role in the biological oceanography of the fjord.[2] Although the relative quantitative contribution of this segment of the phytoplankton population is now known for this area, the qualitative aspects of this difficult group of unicellular organisms are virtually unknown. In the months of January, February, and March, 1960, some preliminary observations were made on samples taken from several depths in Indian Arm and maintained in the laboratory under a variety of cultural conditions. As a result of these observations we have encountered a great variety of microorganisms, many of which present difficult taxonomic problems because of their extremely small size and the paucity of taxonomic literature relating to them. Amongst these, several new entities have been discovered, and these are described here for the first time. In addition, new records of distribution for this area are reported. Diatoms, also present in abundance in the nannoplankton, are excluded from this report.

The Environment

Indian Arm extends due north from a point at 49°18′10″ N. and 122°56′20″ W. for a distance of approximately 22 kilometers. The average width of the fjord is slightly over 1 kilometer. At the entrance there is a shallow sill about 26 meters deep and at the head the fjord shoals gradually into the mouth of the Mesliloet River. In the central region there is a deep basin with a maximum depth of 224 meters and an average depth of 200 meters.

The surface waters of Indian Arm are brackish, ranging in salinity from 1% near the head to 25% at the entrance, but below a depth of about 5 meters

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[2]Gilmartin, M. Ph.D. Thesis, University of British Columbia. J. Fisheries Research Board, Canada (in press).

ranging from 25% to 27%. The surface temperatures during the summer range between 10° and 16° C to a depth of 30 meters and between 6° and 8° C below; and during the winter months between 5° and 7° C near the surface and 6° and 8° C at greater depths. The oxygen distribution is characterized by high surface values, which at times reach 150% saturation and exceptionally as high as 250% (associated with intense phytoplankton blooms) near the surface. These decrease rapidly with depth to values ranging from 10 to 30% near the bottom.

Collection and Culture Methods

Water samples were taken at various depths from the surface to a depth of 150 meters in Indian Arm using a Van Dorn type plastic water bottle (14). After the bottles were closed the samples were brought to the surface and placed in polyethylene storage bottles for transfer to the laboratory.

The original samples were treated in several ways to establish unialgal cultures. In general, enrichment nutrients including nitrates, phosphates, trace elements, and soil extract (see (13), Erdschreiber medium) were added to the samples. The final volume of the culture varied with the size of the container and the size of the aliquot of original sample. The most satisfactory cultures (in quality and quantity) were obtained in 125-ml Erlenmeyer flasks containing 10 ml concentrated sample to which 30 ml of enrichment medium was added. The concentrates were obtained using 47 mm Type AA Millipore filters (0.80 μ pore size). The other techniques employed were: 20 ml aliquot placed in 25-ml screw-cap culture tube, with no enrichment nutrients; enrichment cultures with total volumes of 100 ml (80 ml sample), 200 ml (160 ml sample), and 250 ml (195 ml filtered and sterilized sea water; 10 ml concentrated sample) placed in 250-ml B.O.D. bottles. Some of the B.O.D. bottles were set up in duplicate with one placed in the dark and the other in the light. Duplicate series of cultures were originally exposed to several temperatures (4°, 9–12°, and 22–26° C) but were later all maintained at 9–12° C. The light intensity was approximately 100 foot-candles and the cultures were maintained on a 12 hour light – 12 hour dark period.

Microchemical Techniques

The microchemical techniques included the standard test for starch (Iodine Potassium Iodide), and oil (Sudan III). The cell walls and loricas were tested for cellulose by the standard test (IKI plus 75% H_2SO_4); and the addition of "Shirlastain" (4). The latter reagent, manufactured by the Shirley Institute, stains cellulose a violet-pink (Dr. T. R. Parsons, Fisheries Research Board of Canada, personal communication). The occurrence of pectin was tested by the addition of a 0.001% solution of ruthenium red.

Organisms Encountered

I. CLASS CHRYSOPHYCEAE

Chromulina sp. (Fig. 1)

An undetermined species of the genus *Chromulina* appeared in the cultures from samples collected in February near the head of the fjord. The cells measure 3–5 μ in diameter. The two band-shaped chromatophores are peri-

pheral, each containing several refractile oil droplets. Neither chromatophore contains an eyespot. The single flagellum, which arises from a papilla, is twice the length of the cell.

Ochromonas ?vallesiaca Chodat (Fig. 2)

This species was present in cultures obtained from samples originating near the head of the fjord. The metaboly of the organism is quite marked. The cells measure 5–7 μ in diameter. The one cup-shaped chromatophore contains an anterior eyespot and many refractive granules. The longer flagellum is 15 μ whereas the shorter one is only 7–8 μ in length.

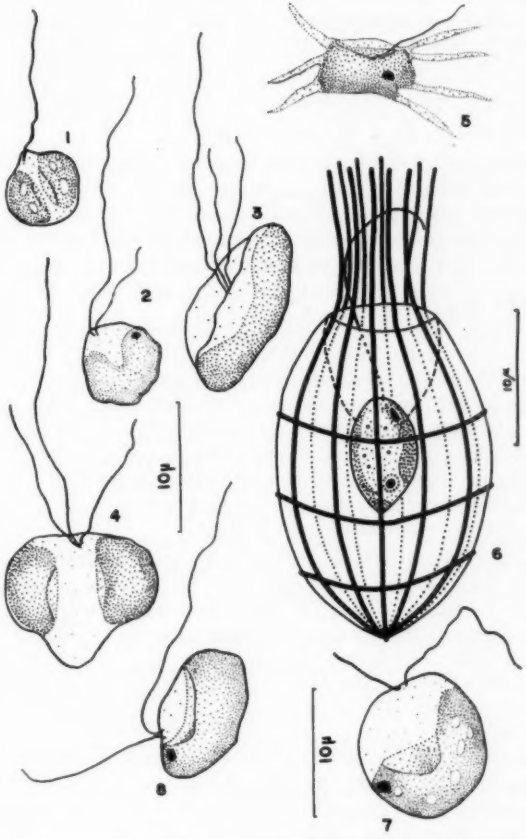


FIG. 1. *Chromulina* sp. FIG. 2. *Ochromonas ?vallesiaca* Chodat. FIGS. 3, 4. *Chrysochromulina* sp. FIG. 5. *Chrysoamoeba nana* sp. nov. FIG. 6. *Microsportella fiordensis* sp. nov. FIGS. 7, 8. *Xanthomonas thalassoides* sp. nov.

Chrysochromulina sp. (Figs. 3, 4)

A species of this genus was observed in several cultures obtained from samples collected near the surface. The cell is 10–12 μ in diameter (at the widest and longest parts) and has two unequal flagella as well as a nonmotile filamentous haptonema. This trilobed flagellate contains two clearly defined yellow-brown chromatophores. As a result of the extensive studies of *Chrysochromulina* by Parke, Manton, and Clarke (8, 9, 10, 11) with the electron microscope, no attempt has been made to determine the species as yet.

Chrysamoeba nana sp. nov. (Fig. 5)

Cellula amoeboidea, 5 μ lat., 3 μ long., pseudopodiis exceptis; chromatophorus unicus flavo-brunneus cellulam complens; stigma medium; guttulae olei nullae; unicum flagellum immobile; motus tardus per pseudopodia tenuia.

Amoeboid; 5 μ wide by 3 μ long, excluding pseudopodia; single yellow-brown chromatophore filling the cell, median eyespot; oil droplets lacking; single flagellum immobile; movement slow and by slender pseudopodia.

Type locality: Indian Arm, British Columbia.

This pigmented amoeboid form occurred in cultures from the station closest to the head of the inlet. The entire cell appeared to be filled with the yellow-brown chromatophore. The flagellum was a little longer than the pseudopodia and difficult to distinguish. Because of the small size of the cell it is impossible to place this taxon in any of the presently recognized species (see (1) and (5)).

Microsportella gen. nov.

Planta unicellularis in lorica hyalina costata; pars dilute viridis parum colorata ovata; 5 μ lat., 8 μ long.; unicum flagellum 15–17 μ long. Protoplastus in lorica sportelliformi perlucida aliquantulum complanata 16 μ lat., 24 μ long. (cum spinis) inclusus; lorica qua in parte flagellum emergit invaginata, contra extremitatem clausam acute attenuata. Lorica tribus costis peripheralibus ad axem longitudinalem transverse dispositis plerumque ornata, necnon 10 costis longitudinalibus emergentibus, ad extremitatem clausam se commiscentibus, ultra extremitatem apertam, autem, ad fimbrias spinarum formandas, per spatium 10 μ discrete extendentibus, ornata.

Unicellular, in hyaline ribbed lorica; faintly pigmented pale green portion ovoid; 5 μ wide by 8 μ long; single flagellum 15–17 μ long. Enclosed in transparent, slightly flattened, basketlike lorica 16 μ wide by 24 μ long (including spines); lorica invaginated where flagellum emerges, tapering to a point opposite closed end. The lorica usually ornamented with three peripheral ridges transverse to longitudinal axis, and 10 emergent longitudinal ridges that merge at closed end but extend freely a distance of 10 μ to form a fringe of spines beyond open end.

The name is derived from the Latin: *micro* = tiny; *sportella* = little basket.

Type species: ***Microsportella fiordensis*** sp. nov. (Fig. 6)

Descriptio eadem ut in genere.

Description same as for the genus.

Type locality: Indian Arm, British Columbia.

The pigment in this tiny flagellate appears to be confined to a peripheral,

laminate chromatophore, and gives the cell a faint pale green appearance. The cytoplasm is granular and several protoplasmic bodies can be seen distributed in the cytoplasm. The largest, which is usually toward the end opposite the flagellum, is thought to be the nucleus. Close to the point of insertion of the flagellum there is a refractive, lenticular body which may be stigmatic. Standard tests for starch and oil were negative. The standard cellulose and pectin tests were negative also; however, with the addition of "Shirlastain" the lorica became violet-pink, indicative of the presence of cellulose. Upon the addition of India ink to the microscopic preparations, the open end of the lorica became filled with ink particles.

Microsportella was rarely seen in the original collections but appeared in cultures from depths of 5, 10, 20, 50, and 150 meters.

The taxonomic position of this genus is uncertain. It appears to be closely related to such colorless genera as *Diaphanoeca* Ellis and *Stephanoeca* Ellis, which are classed with the colorless Choanoflagellata. *Microsportella* differs from *Diaphanoeca* in the number of longitudinal ridges, the markings of transverse ridges, and the lack of gelatin in the lorica, which Ellis (3) believed present. It differs from *Stephanoeca* in form and in color of lorica. Bourrelly (1) does not recognize *Diaphanoeca* but places it in with *Stephanoeca*. He considers these unpigmented forms to be members of the Chrysophyta because of cyst (statospore) formation by certain colorless genera, and the occurrence of two yellow-brown chromatophores in a 'typical' choanoflagellate, *Stylochromonas* described by Lackey (5) from the Woods Hole, Massachusetts, area. It is possible that *Microsportella* is another "connecting link" between the pigmented Chrysophyceae and the colorless Choanoflagellata and closer to the Choanoflagellata than *Stylochromonas*.

II. CLASS XANTHOPHYCEAE

Xanthomonas gen. nov.

Planta unicellularis; spherica, 10 μ diam., unicus chromatophorus poculiformis dilute flavo-viridis dimidiam cellulae partem stigma in chromatophoro anterius; pyrenoidea nulla; oleum ut guttulae coacervatum; flagella duo imparia; flagella breviore 10 μ longiore, 25 μ long.

Unicellular; spherical; 10 μ in diameter; single pale yellow-green cup-shaped chromatophore half filling the cell; eyespot anterior in chromatophore; lacking pyrenoids; stores oil as droplets; biflagellate, unequal, short flagellum 10 μ, long one 25 μ in length.

The name is derived from the Latin: *xantho* = yellow; *monas* = single.

Type species: ***Xanthomonas thalassoides*** sp. nov. (Figs. 7, 8)

Descriptio eadem ut in genere.

Description same as for the genus.

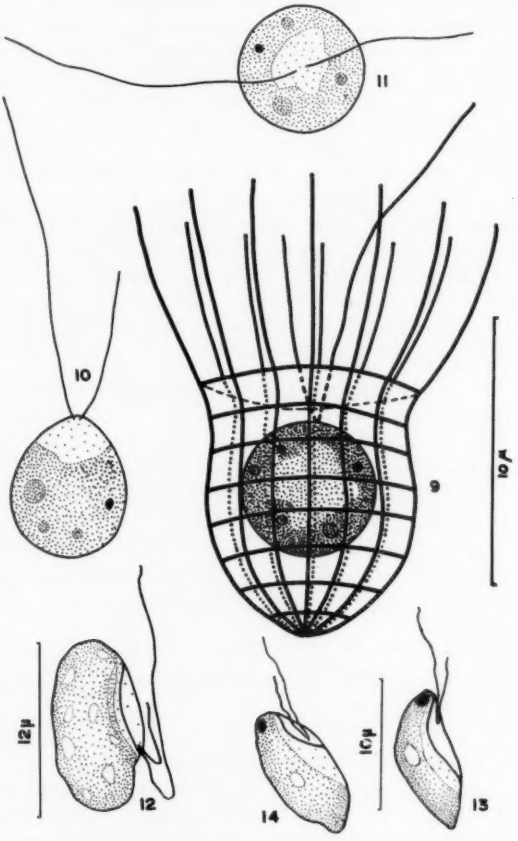
Type locality: Indian Arm, British Columbia.

This alga appeared in cultures from the February collection at a depth of 5 meters near the head of the fjord. Its occurrence in natural populations has not, as yet, been reported. It bears superficial resemblance to Butcher's (2) genus *Thalassomonas*. That it probably belongs to the Xanthophyceae was determined by its color and negative starch test. Motile members of this class

are of rare occurrence. This taxon may be considered as a prototype of the flagellated cells of the nonmotile forms in the fresh-water habitat.

Pseudomicrosportella gen. nov.

Planta unicellularis in lorica hyalina costata saepe reperta; pars parum colorata spherica; 5 μ diam.; biflagellata, heterokontea; flagello breviore 7 μ, longiore 14 μ. Protoplastus in lorica sportelliformi perlucida 7–5 μ lat., 10 μ long. (cum spinis) saepe inclusus; lorica ad extremitatem clausam attenuata, ad extremitatem apertam ora parva praedita. Lorica 7 costis periferalibus emergentibus, ad axem longitudinalem transverse dispositis, plerumque ornata, necnon 12 costis longitudinalibus emergentibus ad extremitatem clausam se



FIGS. 9–11. *Pseudomicrosportella ornata* sp. nov. (9, loricated form; 10, zoospore, lateral view; 11, zoospore, polar view). FIG. 12. *Anisomonas astigmatica* sp. nov. FIGS. 13, 14. *Thalassomonas exurgens* Butcher.

commiscentibus, ultra extremitatem apertam, autem, ad fimbrias spiniformes formandas, per spatium 12 μ discrete extendentibus, ornata.

Unicellular, often in hyaline ribbed lorica; faintly pigmented portion spherical; 5 μ in diameter; biflagellate, heterokont, shorter flagellum 7 μ, longer one 14 μ in length. Often enclosed in transparent, basketlike lorica, 5–7 μ wide, 10 μ long (including spines); lorica tapers at the closed end, slightly flanged at open end. Lorica usually ornamented with 7 emergent peripheral ridges, transverse to the longitudinal axis; and 12 emergent longitudinal ridges that merge at closed end but extend freely for a distance of 12 μ to form a spinelike fringe beyond open end.

The name is derived from the Latin: *pseudo* = false; *microsportella* = tiny little basket.

Type species: ***Pseudomicrosportella ornata*** sp. nov. (Figs. 9, 10, 11)

Descriptio eadem ut in genere.

Description same as for the genus.

Type locality: Indian Arm, British Columbia.

This species is known in two stages: one as described above in the lorica, and the other as a free-flagellated cell (zoospore?) (Figs. 10, 11). The pigmented portion appears the same in its shape and cytoplasmic characteristics in both phases. The pigment appears to be confined to a peripheral laminate chromatophore and gives the cell a distinctly yellowish-green appearance. The cytoplasm is granular and several protoplasmic bodies, varying in density, can be seen distributed in the cytoplasm and the chromatophore. Droplets of oil are detectable in the cytoplasm. Standard test for starch was negative, but a positive test for oil was obtained. The cellulose test for the cell wall was also negative. The composition of the lorica is unknown, as all tests for cellulose and pectin were negative. Upon the addition of concentrated HCl, the lorica became less distinct, possibly indicating a calcareous nature.

Pseudomicrosportella was also rarely seen in the original samples. Loricated forms were cultured from depths of 0, 5, 20, 50, and 100 meters and the zoospore forms from all but the deepest and including 10 meters.

The taxonomic position of this species is also somewhat uncertain, but from the heterokont flagellation, yellow-green pigmentation, and the positive oil test, it is considered to be a member of the Xanthophyceae.

III. CLASS CHLOROPHYCEAE

Anisomonas astigmatica sp. nov. (Fig. 12)

Planta unicellularis nuda; ovata ad extremitatem anteriorem complanata; 6 μ long., 10 μ lat.; unicus chromatophorus poculiformis cellulam implens, pyrenoideum stigmaque nulla; chromatophorus granulis hyalinis impletus, amylum ignotum; flagella duo imparia ex extremitate anteriore complanata excentrice enascentia, breviore iuxta cellulam, alterotriplo longiore manifesto.

Unicellular; naked; ovate, flattened at anterior end; 6 μ long by 10 μ wide; single cup-shaped chromatophore fills the cell, lacking pyrenoid, lacking eyespot; chromatophore filled with hyaline granules, occurrence of starch unknown; biflagellate, unequal, arise excentrically from flattened anterior end, shorter one close to cell, longer flagellum obvious and three times as long.

Type locality: Indian Arm, British Columbia.

This new species in Butcher's genus (2) differs from the type, *Anisomonas longifilis* Butcher, by the length of the flagella, the size of cell, and absence of an eyespot. The swimming movement of *A. astigmatica* is more regular than that of the type, which may mean that the flagella are "isodynamic." If this is true, then this taxon might be a new genus, according to Butcher's (2) delimitation of *Anisomonas* (flagella unequal, simple, heterodynamic). The exact meaning of the terms "isodynamic" and "heterodynamic" as used by Butcher is unknown. Until further studies are made, it seems best to leave it as *A. astigmatica*.

Thalassomonas exurgens Butcher (Figs. 12, 13)

This species was fairly abundant in a culture incubated 1 month in the dark at 4° C. It was a very pale green in color and appeared to be light-sensitive, as noted by Butcher (2). The single cup-shaped chromatophore contained an anterior eyespot and a basal pyrenoid. No starch test was made. The entire cell was 3 μ wide by 10 μ long. The two unequal flagella arise from an excentric infolding close to the eyespot. Although the culture was not unialgal, *T. exurgens* appeared fairly abundant. Ordinary light culture techniques may account for the apparent difficulty encountered by Butcher (2) with his isolate, since this appears to be a heterotrophic form.

Butcher (2) separated the starch-containing yellow-green nannoplankton on the basis of flagellation. This included the relative length, presence or absence of mastigonemes, and type of movement (isodynamic vs. heterodynamic). As mentioned above, the exact meaning of these terms is not defined by Butcher (2). He placed these taxa in the Chlorophyta as a matter of convenience. The importance of any one morphological criterion (such as storage products, pigmentation, flagellation) has not been determined. It may be that information on the pigments of both *Anisomonas* and *Thalassomonas* will place them more precisely in the Xanthophyceae. Similarly, information about the chlamydomonad *Platymonas* (*Tetraselmis* in Butcher (1)) may show it too has affinities with the Xanthophyceae, particularly since it has mastigonemes on the flagella (12), and noncellulosic cell walls (7).

This study is being continued to determine qualititatively the nannoplankton populations in Indian Arm, and an attempt is also being made to explain their distribution seasonally and three-dimensionally in the fjord in relation to the physical–chemical aspects of the environment.

Acknowledgments

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References

1. BOURRELLY, P. 1957. Recherches sur les Chrysophycées: morphologie, phylogénie, systématique. Rev. Algol. Mem. Hors-Ser. 1. pp. 1–412.

2. BUTCHER, R. W. 1959. An introductory account of the smaller algae of British coastal waters. Part I: Introduction and Chlorophyceae. Fishery Investigations Ser. IV, Ministry of Agriculture, Fisheries, and Food.
3. ELLIS, W. N. 1929. Recent researches on the Choanoflagellata (Craspedomonadines). Ann. soc. roy. zool. Belg. **60**, 49–88.
4. HEYN, A. N. J. 1954. Fiber microscopy. Interscience, New York. pp. 252–253.
5. HUBER-PESTALOZZI, G. 1941. Das Phytoplankton des Süsswassers. *In* A. Thienemann, Die Binnengewässer. Bd. 16(2), 1 Hälfte. E. Schweizerbart, Stuttgart.
6. LACKEY, J. B. 1940. Some new flagellates from the Woods Hole area. Am. Midland Naturalist, **23**, 463–471.
7. LEWIN, R. 1958. The cell walls of *Platymonas*. J. Gen. Microbiol. **19**, 87–90.
8. PARKE, M., MANTON, I., and CLARKE, B. 1955. Studies on marine flagellates. II. Three new species of *Chrysochromulina*. J. Marine Biol. Assn. U. K. **34**, 579–609.
9. PARKE, M., MANTON, I., and CLARKE, B. 1956. *Ibid.* III. Three further species of *Chrysochromulina*. J. Marine Biol. Assn. U. K. **35**, 387–414.
10. PARKE, M., MANTON, I., and CLARKE, B. 1958. *Ibid.* IV. Morphology and microanatomy of a new species of *Chrysochromulina*. J. Marine Biol. Assn. U. K. **37**, 209–228.
11. PARKE, M., MANTON, I., and CLARKE, B. 1959. *Ibid.* V. Morphology and microanatomy of *Chrysochromulina strobilus* sp. nov. J. Marine Biol. Assn. U. K. **38**, 169–188.
12. PITELKA, D. and SCHOOLEY, C. 1955. Comparative morphology of some protistan flagella. Univ. Calif. Publ. Zool. **61**, 79–128.
13. STARR, R. C. 1960. The culture collection of algae at Indiana University. Am. J. Botany, **47**, 67–86.
14. VAN DORN, W. G. 1956. The large-volume water sampler. Trans. Am. Geophys. Union, **37**, 682–684.

NEW SPECIES OF THE GENUS CERATOCYSTIS[1]

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Abstract

A study of the species of *Ceratocystis* found in Ontario on decaying slash has been made and most of the species grown in pure culture. The characteristics of the genus and the separation of species are discussed. Four species found on wood of coniferous trees are described as new. Illustrations are included for all of these. *Ceratocystis brunneocrinita* Wright and Cain is described from several collections of *Abies balsamea*. The perithecia have brown ostiolar hyphae and the ascospores appear rectangular. *Ceratocystis europhioides* Wright and Cain, on *Pinus* and *Picea*, has ascospores similar to but larger than the nonostiolate *Europhium trinacriforme* Parker. *Ceratocystis falcata* Wright and Cain, on *Pinus*, resembles *Ceratocystis minuta* (Siem.) Hunt but is distinguished by the longer, septate ascospores. *Ceratocystis sagmatospora* Wright and Cain, on *Pinus* and *Picea*, has saddle-shaped ascospores with elaborate gelatinous sheaths.

Introduction

Fungal discoloration of sapwood of conifers, generally diagnosed as blue-stain, is caused by several different fungi possessing dark-colored hyphae, many of which belong to the genus *Ceratocystis*. Not all of the species of *Ceratocystis* growing on conifers are economically important blue-staining fungi, but all may be found, at one time or another, associated with insects which are the agents for the efficient dispersal of the spores. The numerous investigations of fungi belonging to this genus have recently been reviewed in an excellent manner by Mathiesen-Käärik (1960), and will not be repeated here.

The present paper describes four new species of the genus *Ceratocystis* which were isolated in connection with a study of slash deterioration in Ontario. Especially notable in these interesting fungi are the ascospores, which in some species are enclosed by an elaborate gelatinous sheath.

Materials and Methods

Cultures were obtained from fresh material collected in the field. The ascospore-ooze which collected at the tip of the neck was transferred to agar slants. Leonian's + 0.1% yeast extract agar was predominantly used in the cultural studies. For some species, a drop of lactic acid, added to the slant 2 days before transfer of the fungus, provided a better medium for the production of the perfect stage. This was especially effective in the case of *Ceratocystis europhioides* sp. nov.

Perithecia were observed in crushed water mounts. Semipermanent mounts were made by placing a drop of cotton blue in lactophenol at one side of the cover slip. Occasionally the slides were first flooded with 2% KOH. With

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[3]Department of Botany, University of Toronto.

this technique, it was found that the asci expanded, the ascospores separated out more readily, and their gelatinous sheaths became enlarged. All measurements were made from water mounts. The dimensions given for ascospores did not include the gelatinous sheath since the diameter of this component varies with the age and condition of the culture as well as with moisture content. Drawings were made from water mounts with the aid of a camera lucida unless otherwise specified.

The Genus *Ceratocystis* and the Separation of Species

Hunt (1956) has given a formal generic diagnosis and review of *Ceratocystis*. The members included in the genus are characterized by having perithecia with brown to black bases, elongate ostiolated black necks, irregularly disposed, evanescent asci, and with hyaline, unicellular ascospores. Variations in these characters in conjunction with the structure of imperfect stages as well as cultural features have been utilized to distinguish species and to indicate relationships within the genus.

The concept of the genus is based on the type *Ceratocystis fimbriata* Ellis and Halsted (in Halsted and Fairchild 1891). In this species there is present a dark color in the cells of the long, cylindrical neck and in the outer pseudoparenchyma cells of the globose perithecial base. Color develops with age and may vary somewhat in mature perithecia. *C. sagmatospora* sp. nov. and *C. minuta* (Siem.) Hunt often have perithecia with light brown bases by transmitted light. *C. bicolor* (Davidson and Wells) Davidson is an exceptional species in which the base remains light-colored as in species of *Melanospora*. The dark color of the perithecia is probably of generic significance; the lighter-colored forms represent an expression of a slight physiological variation.

In some species the bases of the perithecia are curiously ornamented. In *C. moniliformis* (Hedgecock) C. Moreau, the spines are short, conical, and dark. Some species have brown, septate hairs; others may have undifferentiated hyphae. The ornamentation seen in these species of *Ceratocystis* differs from the setaelike hairs found occurring on the perithecia of the related genera *Microascus* Zukal and *Petriella* Curzi.

In *Ceratocystis fimbriata* Ellis and Halsted, the slender neck consists of upright, laterally fused, septate filaments surrounding a central pore. Not all species of *Ceratocystis* have a neck of this type. In some, the neck filaments develop into rough pseudoparenchyma as in *C. penicillata* (Grosm.) C. Moreau and *C. europhioides* sp. nov. In our collections, *C. minuta* has a short, broad neck and the related species, *C. falcata* sp. nov., has a conical neck. This extreme variability in the length of the necks, within the present concept of species limits, has been recorded in the literature. In similar species the range overlaps so that descriptions including only the longest dimension may be misleading. On wood and acidified media *C. europhioides* sp. nov. produces elongated necks while on modified Leonian's agar no necks were produced or only short papillae were observed even on those perithecia containing mature ascospores. *C. europhioides* sp. nov. can be separated from *Europhium trinacriforme* Parker (1957) primarily on the basis of the nature of the neck. A neck is lacking in *Europhium trinacriforme*. The appearance of a neck may be useful as a generic and as a specific character but only when other features

are also considered.

In many species of *Ceratocystis*, the filaments of the neck separate at the apical end to form ostiolar hyphae while the structures around the ostiole in *Microascus* and *Petriella* appear to be modifications of the external, setaelike structures. Several species of *Ceratocystis* have hyaline, flaring, ostiolar hyphae, but other species have erect or spirally curved brown structures. Lagerberg, Lundberg, and Melin (1927) pointed out that the form and number of the ostiolar hyphae may be good species characters but should not be interpreted as deciding features in themselves. Certainly, such distinctive, short, conically arranged, ostiolar hyphae as seen in *C. minuta* are an excellent delineating feature.

Cytological investigations (Elliott 1925; Sartoris 1927; Varitchak 1931; Mittman 1932; Andrus and Harter 1933 and 1937; Andrus 1936; Gwynne-Vaughan and Broadhead 1936; Taylor-Vinje 1940; Hutchinson 1950; Bakshi 1951; M. and Mme. Moreau 1952) on the development of perithecia, in several species, have revealed different interpretations as to the development of ascogenous cells and the formation of asci. Although some descriptions indicate that the asci are connected in chains and others show that they are irregularly disposed, there is general agreement that the asci occur throughout the sterile pseudoparenchyma tissue. Nannfeldt (1932) described the asci as "*rundlichen*" for the species that he included in *Ophiostoma* (in Melin and Nannfeldt 1934). Luttrell (1951) described the *Ophiostoma*-type of ascus as globoid and nonstipitate. Illustrations of young asci, in many of the previously cited cytological papers, have shown them to be distinctly clavate. The present authors have observed clavate asci from young perithecia and gelatinous remnants of the asci surrounding the subglobose spore masses in mature perithecia. In *C. europhioides* (Fig. 10) and in *C. bicolor* the broadly clavate asci appeared to have a fasciculate arrangement. The evanescent asci which provide the gelatinous matrix of the spore masses are a specialization for insect dispersal. This mechanism is more efficient when the asci are dispersed throughout the perithecial cavity. Perhaps evolution of asci in species of *Ceratocystis* is proceeding toward a completely irregular arrangement of globose, evanescent asci; however, many species still show transitional stages in the shape and disposition of these structures.

In many genera of Ascomycetes the ascospore is regarded as the fundamental character for the separation of species. Perhaps it is because of the small sizes of the ascospores that a number of the species of *Ceratocystis* have their spores described as either bean-shaped or as sections of an orange. Accurate illustrations of ascospores to substantiate descriptions are invaluable but often lacking in the literature. In the genus there are ascospores of a variety of shapes, some of which are surrounded by curious, gelatinous sheaths. The ascospores which have a sheath are particularly interesting when all three views are observed. The hyaline ascospores emerge from the ostiole embedded in a hyaline to light yellowish-brown gelatinous matrix which forms either a viscid droplet or a cirrhus. Although all other species of the genus have one-celled ascospores, *C. falcata* has septate spores, but is being placed in *Ceratocystis* for the time being.

In addition to the characteristics of the perfect stage, the imperfect stages

have been utilized to separate species and to indicate relationships. The genera *Endoconidiophora* Münch and *Grosmannia* Goid. were founded on the imperfect stages but have since been rejected. The imperfect stages have been referred to several genera, among which have been *Cephalosporium* Corda, *Cladosporium* Link ex Fries, *Leptographium* Lagerb. and Melin, *Graphium* Corda, *Chalara* Corda, and *Thielaviopsis* Went. These have been discussed at some length by Münch (1907); Lagerberg, Lundberg, and Melin (1927); Robak (1932); Melin and Nannfeldt (1934); Mathiesen-Käärik (1953); and Hunt (1956).

Three basic types of conidial formation have been observed in species of *Ceratocystis* by the present authors. In the first and most easily recognized type the conidia originate as "endoconidia". The production of "mycelial conidia" appears to be highly variable. Actually most of these conidia are constricted from the terminal apex of hyaline hyphae which then grow outwards at the base of the conidium to form a new hyphal apex. This may elongate or produce a new conidium. In this way, irregular, wavy conidiophores are produced. If little elongation of the hypha occurs and new conidia are produced, several conidia may become aggregated into a watery head resembling that produced in *Cephalosporium*. In some instances the conidia are broadly attached to the conidiophores and in others a very narrow connection leaves denticles on the conidiophores. These conidia may remain attached to the conidiophore, develop thicker walls, and produce secondary conidia. In some species, a third type of conidia or phialospores is produced on phialides. The phialides are found at the ends of rather specialized hyaline or brown, branching conidiophores. Phialides are observed most clearly in *C. sagmatospora*, which tends to form coremia. Phialospores are also produced in *C. europhioides* which has the *Leptographium*-type of imperfect stage. No comprehensive study of conidial formation in all species of *Ceratocystis* or related imperfect genera was undertaken, but it is suggested that the exact method of origin of conidia may be a more significant feature than that of the presence or absence of single conidiophores or coremia.

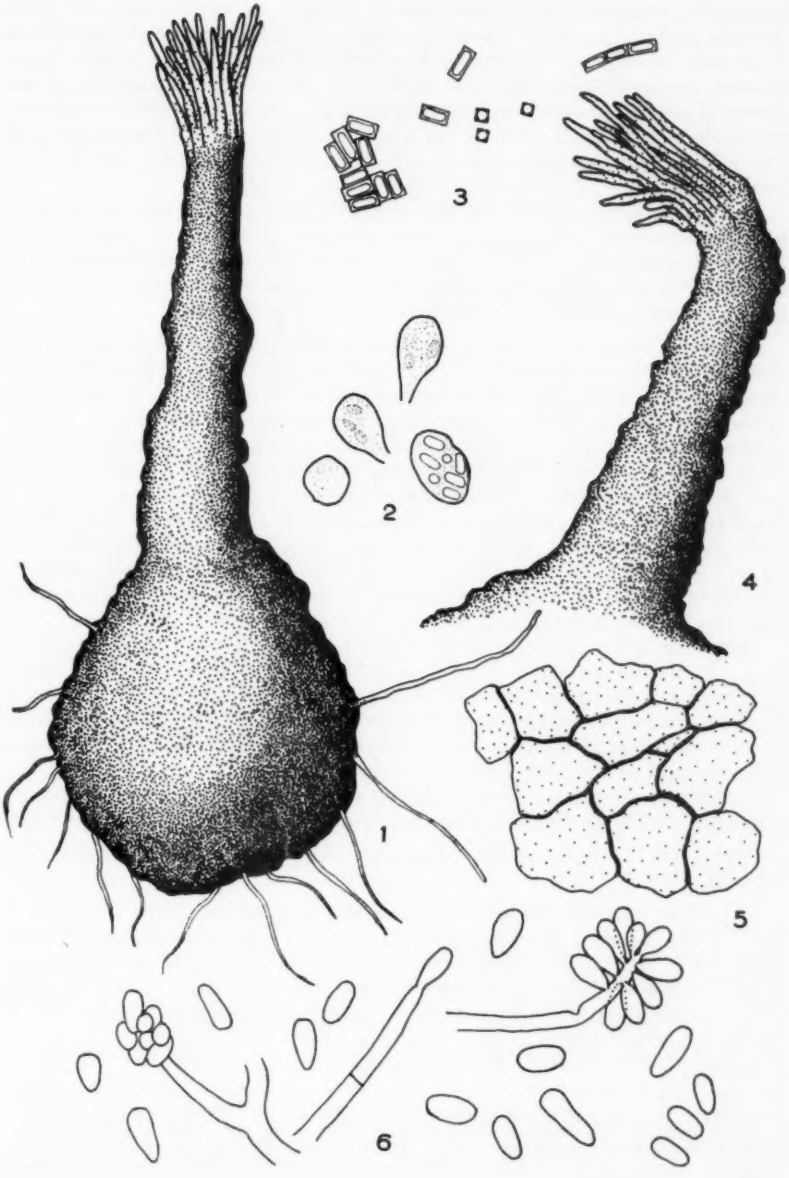
At the present time, the shape and size of the perithecium, the ascospore, and the gelatinous sheath (when present) together with imperfect and cultural characteristics are all required for the identification of species. It is believed that with adequate illustrations and descriptions of perithecia and ascospores positive identification can be made from wood collections alone.

Descriptions of Species

Ceratocystis brunneocrinita Wright and Cain sp. nov. (Figs. 1–6, 21)

Peritheciis in cultura et in ligno superficialibus vel immersis, nigris subglobosis, 70–150 μ diam.; peridio cellularum tenus 16 μ diam.; collo saepe curvo, nigro, longo 100–450 μ, basi 20–40 μ, 11–25 μ sub hyphis ostioli, ex fibris multiseptatis composito; hyphis ostioli brunneis, leviter digredientibus,

FIGS. 1–6. *Ceratocystis brunneocrinita*. Fig. 1. Perithecium from a culture, TRTC 34581b, ×430. Fig. 2. Asci in various stages of development, TRTC 34581b, ×1200. Fig. 3. Ascospores in lactophenol, TRTC 34543, ×1200. Fig. 4. Perithecial neck from a culture, TRTC 34581b, ×430. Fig. 5. Peridial cells in lactophenol, TRTC 33379, ×1200. Fig. 6. Conidiophores and conidia in culture, TRTC 34543, ×1700.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latitudine irregularibus et apice hebeti ornatis, 10 vel 30 numero, longis 25–55 μ × 2.0–3.5 μ diam. Ascis clavatis, 9.5×5.5 μ, dilatatis vel subglobosis et in aqua dissolutis, irregulariter dispositis. Ascosporis hyalinis, una cellula ornatis, aliquando biguttulatis, a fronte et a latere conspectis angulum rectum formantibus, sed oblongis in lactophenol, vagina hyalina et angulum rectum formante circumventis, 3.0–4.5 × 1.0–2.0 μ (vagina non computata), 3.5–5.0 × 1.4–2.4 μ (vagina addita), ad apicem colli conglobatis sed in aqua facile dispersis.

Cultura in agaro constanter alba; hyphis aeriis paucis, mycelio hyalino, hyphis angustis 1.2–1.8 μ ornato. Solum paucis peritheciis in cultura in parte superiore agari ortis. Conidiis hyalinis, una cellula praeditis, ellipsoideis vel ovoideis, 3.2–6.0 × 1.2–2.0 μ, paucis numero et circum ultimas partes hypharum ab reliquo mycelio vix distinctarum uvas formantibus.

Perithecia in culture and on wood, superficial or embedded, black, subglobose, 70–150 μ in diameter. Cells of peridium up to 16 μ in diameter. Neck often curved, black, 100–450 μ long, 20–40 μ wide at base, 11–25 μ wide below the ostiolar hyphae, composed of multiseptate filaments. Ostiolar hyphae brown, diverging slightly, irregular in width with a blunt apex, 10 to 30 in number, 25–55 μ long by 2.0–3.5 μ in diameter. Asci clavate, 8-spored, 9.5×5.5 μ, expanding to subglobose and rapidly disintegrating in water, irregularly disposed. Ascospores hyaline, unicellular, sometimes 2-guttulate, rectangular in face view, and side view, but oblong in lactophenol, surrounded by a rectangular, hyaline sheath, 3.0–4.5 × 1.0–2.0 μ (without sheath), 3.5–5.0 × 1.4–2.4 μ including sheath, collecting in a mass at the apex of the neck but readily dispersing in water.

Colony on agar remaining white with little aerial growth. Mycelium hyaline, with narrow hyphae 1.2–1.8 μ in width. Only a few perithecia maturing in culture on the upper end of the agar slant in tubes containing bacteria. Conidia hyaline, unicellular, ellipsoid to ovoid, 3.2–6.0 × 1.2–2.0 μ, produced in small numbers in clusters about the tips of hyphae, hardly differentiated from the rest of the mycelium.

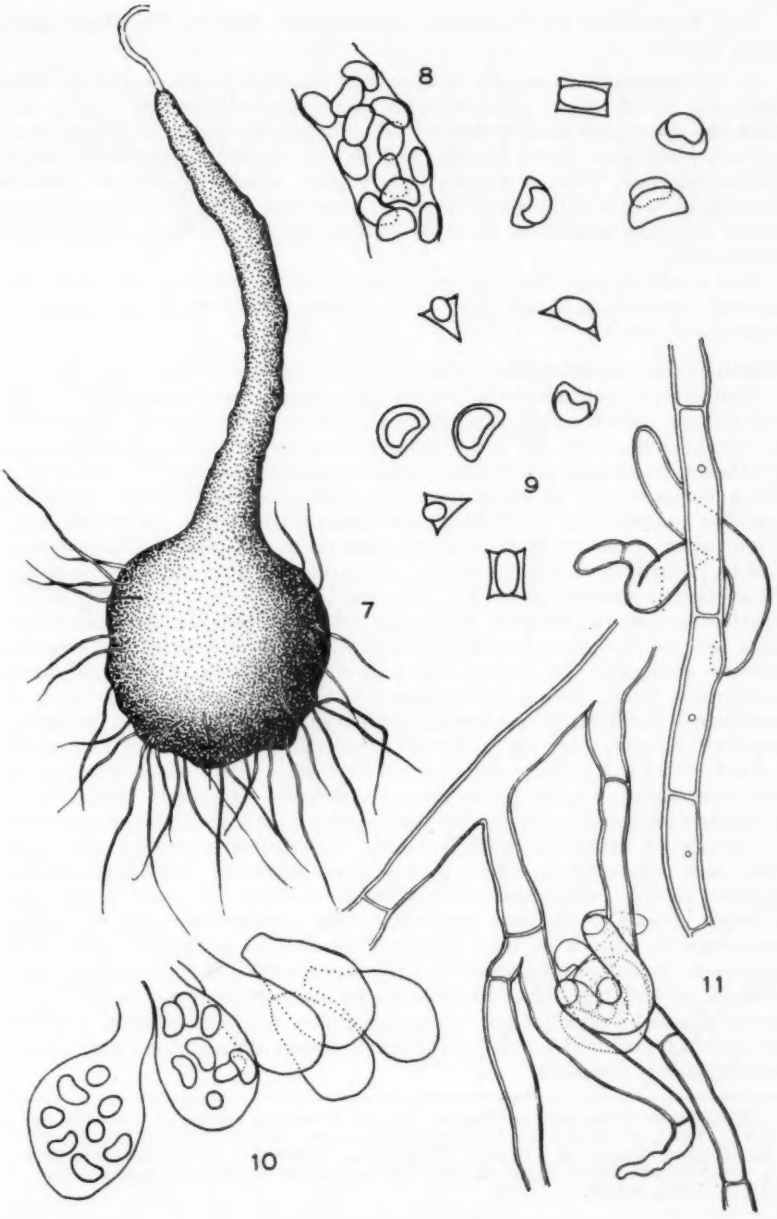
Perithecia were found on the sapwood usually associated with bark beetle tunnels and on bark shreds of *Abies balsamea*. Discoloration was not observed.

Type.—TRTC 34581, Lake Sasajewun, Algonquin Park, Nipissing Co., Ontario, May 30, 1959.

Collections.—Ontario: TRTC 33379, NW. of Nobleton, York Co., July 1, 1957; TRTC 34543, Lake Sasajewun, Algonquin Park, Nipissing Co., May 30, 1958; TRTC 35697, W. of Priceville, Grey Co., May 19, 1958 (all in culture). TRTC 33704, 16 miles N. of Port Severn, Muskoka Dist., May 31, 1958; TRTC 33774, S. of Denbigh, Lennox & Addington Co., Sept. 17, 1958; TRTC 34561, S. of Ballinafad, Halton Co., June 14, 1958.

Quebec: TRTC 34646, SE. of Coaticook, June 11, 1959.

FIGS. 7–11. *Ceratocystis europhioides*. Fig. 7. Mature perithecium from a culture, TRTC 33700, ×120. Fig. 8. Portion of cirrhus from a culture, TRTC 33700, ×1200. Fig. 9. Ascospores in lactophenol, TRTC 33700, ×1200. Fig. 10. Asci swollen in KOH from a culture, TRTC 33700, ×1200. Fig. 11. Perithecial initials from a culture, TRTC 34602, ×1200.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New Hampshire: TRTC 34623b, Ammonoosuc Ravine, Mt. Washington, June 9, 1959.

C. brunneocrinita shows two variations in the form of the perithecia; those occurring in the insect galleries, with long necks and ostiolar hyphae and with the ascospores usually without oil globules, and those perithecia found on bark which were usually smaller, with shorter necks, and without developed ostiolar hyphae. The ascospores were slightly narrower with oil globules towards each end of the spore. Since no consistent characteristics have been found to justify separating these forms, they are considered as variations of one species.

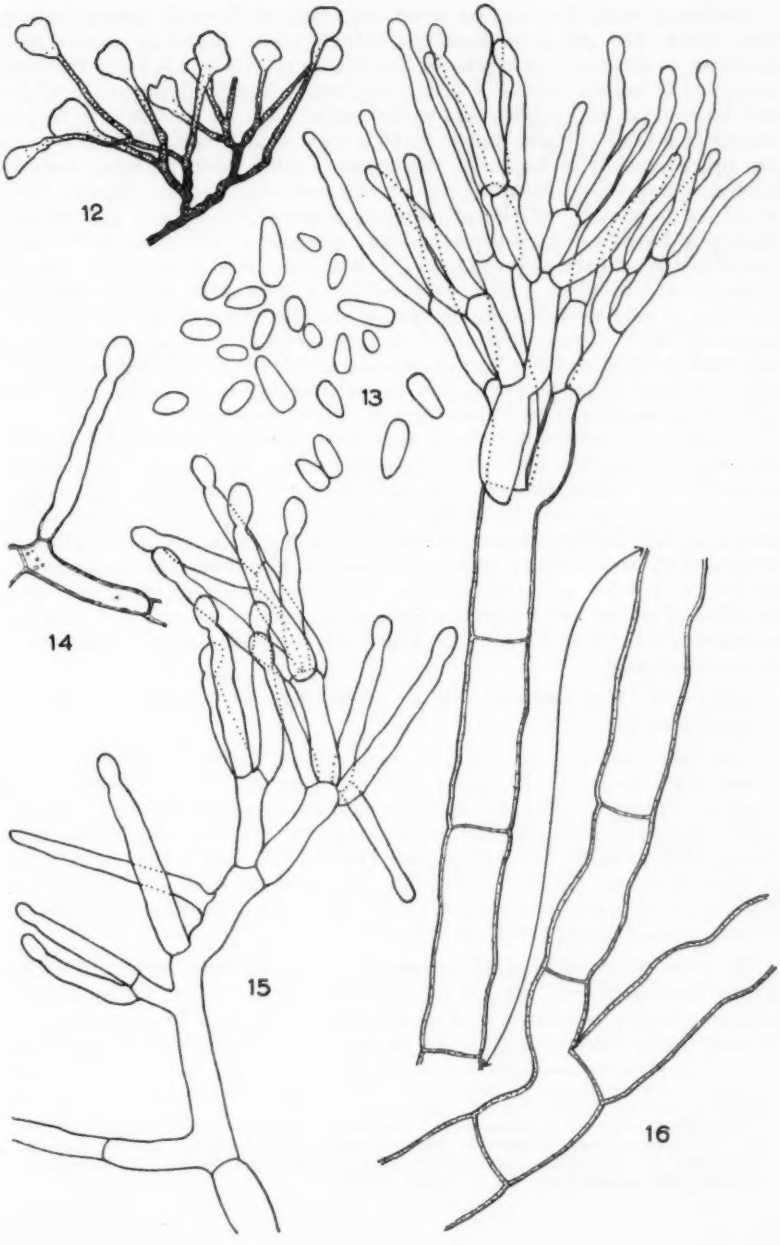
The relationship of this species to others of the genus is not clear. The ostiolar hyphae are darker than in any other species while the ascospores resemble *C. ips*.

Ceratocystis europhioides Wright and Cain sp. nov. (Figs. 7–16, 22)

Peritheciis in cultura et in ligno vel congregatis, subglobosis, nigris, 180–360 μ diam, pilis flexuosis, attenuatis, septatis, basi brunneis et ad ultimam partem hyalinis, tenus 370 × 3.0–4.5 μ ornatis; peridio ex compluribus stratis cellularum nigrarum, exteriorum, multis lateribus praeditarum, tenus 13× 7.0 μ composito et ex strato interiore cellularum hyalinarum, parietibus tenuibus praeditarum composito; collo longo tenus 950 μ, curvo vel recto, nigro, aspero, basi lato 40–60 μ, ad ultimam partem 18–30 μ, excellulis irregularibus, brevibus, 5.0–6.0 μ diam. composito; hyphis ostioli absentibus. Ascis clavatis novellis, postea late clavatis, 15–17 × 11–13 μ (in 2% KOH), evanidis, octosporis, dilatatis et in aqua celeriter dissolutis. Ascosporis hyalinis, una cellula ornatis, reniformibus, concavis a latere conspectis, ovatis a termino conspectis, late ellipsoideis a fronte conspectis, vagina gelatinosa cucullata a latere conspecta, quadrangulata et duobus lateribus longis et contiguis et duobus lateribus brevibus et contiguis ornata a termino conspecta, angulum rectum formante a fronte conspecta partim cinctis; ascosporis 4.5–6.0 × 2.4–3.5 μ (lato magnitudo 2.8–4.0 μ) a latere conspectis (vagina non computata); vagina tenus 4.0 μ prominente; ascosporis emergentibus et cirrhum formantibus, in matrice gelatinosa in aqua lente dissoluta immersis.

Culturis in Leonian's agaro brunneis; hyphis aeriis paucis; peritheciis colla raro formantibus, solum paucis conidiophoribus (similibus conidiophorum generis *Leptographii*) circumventis. Culturis in agaro acido atro-olivaceis, postea atrioribus; peritheciis colla formantibus; conidiophoribus numerosis et singulis et uvas formantibus. Hyphis parietibus atris tenus 13 μ diam. ornatis conidiophoribus atris, septatis 60–300 × 2.2–3.4 μ, ramis hyalinis, penicillatis, saepe unilateralibus ad ultimam partem ornatis. Phialosporis ellipsoideo-ovoideis, ad unum terminum saepe attenuatis, hyalinis, una cellula praeditis, 3.2–8.5 × 2.2–3.4 μ, in massis albis, glutinosis ad apicem conidiophorum dispositis.

FIGS. 12–16. *Ceratocystis europhioides*. Fig. 12. Branching system of conidiophores, TRTC 34602, ×120. Fig. 13. Conidia from a culture, TRTC 33700, ×1200. Fig. 14. Conidiophore directly on a hypha, TRTC 34602, ×1200. Fig. 15. Conidiophore from a young culture, TRTC 33700, ×1200. Fig. 16. Mature conidiophore from a culture, TRTC 34602, ×1200.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Perithecia in culture, and on wood, occurring singly or in groups, subglobose, black, 180–360 μ in diameter, with flexuous, tapering, septate hairs brownish at the base and hyaline at the tip, up to 370 × 3.0–4.5 μ. Peridium composed of several layers of black, polyhedral outer cells up to 13×7.0 μ and inner layer of hyaline, thin-walled cells. Neck up to 950 μ in length, curved or straight, black, rough, 40–60 μ wide at the base, 18–30 μ wide at the tip, composed of irregular, short cells 5.0–6.0 μ in diameter. Ostiolar hyphae absent. Asci clavate when young, becoming broadly clavate, 15–17 × 11–13 μ (in 2% KOH), evanescent, 8-spored, expanding and quickly dissolving in water. Ascospores hyaline, unicellular, the spore itself being reniform, the concave surface showing in side view, oval in end view, broadly ellipsoid in face view, partly enclosed by a gelatinous sheath appearing cucullate in side view, quadrangular with two adjoining long sides and two adjoining short sides in end view, rectangular in face view; dimensions in side view 4.5–6.0 × 2.4–3.5 μ (in extreme width 2.8–4.0 μ) without sheath; sheath projecting up to 4.0 μ. Ascospores emerging in a cirrhus, embedded in a gelatinous matrix that disintegrates slowly in water.

Cultures on modified Leonian's agar brownish with little aerial growth, dotted by perithecia which seldom produce necks, with only a few conidiophores of the *Leptographium* type surrounding the perithecia. Cultures on acidified agar deep olive, becoming darker, with perithecia developing necks, with abundant *Leptographium* conidiophores occurring singly and in clusters. Hyphae with dark walls up to 13 μ in diameter. Conidiophores dark, septate, 60–300 × 2.2–3.4 μ, terminating in hyaline penicillate, often unilateral branches. Phialospores ellipsoid–ovoid, often pointed at one end, hyaline, unicellular, 3.2–8.5 × 2.2–3.4 μ, in white mucilaginous masses at the apex of the conidiophores.

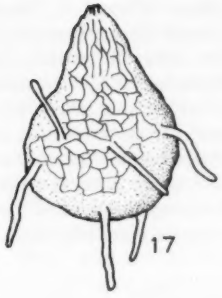
Type.—On *Picea mariana*, TRTC 33700, NW. of Nobleton, York Co., Ontario, May 10, 1958.

Collections.—Ontario: on *Pinus resinosa*, TRTC 33708 and 34668a, Vivian Forest, York Co., June 10, 1958; on *Pinus sylvestris*, TRTC 34601, SE. of Durham, Grey Co., May 17, 1958 (in culture); on *Picea mariana*, TRTC 34602, Algonquin Park, Haliburton Co., May 18, 1959 (in culture); on *Pinus strobus*, TRTC 34658b, W. of Pontypool, Durham Co., Oct. 4, 1958 (in culture); on *Pinus resinosa*, TRTC 34657b, NE. of Mansfield, Dufferin Co., Nov. 8, 1958 (in culture); on *Pinus glauca*, TRTC 36278, Twp. 4F, Mashagama Lake, Algoma Dist., June 17, 1960 (in culture).

The close relationship of *C. europhioides* to *Europhium trinacriforme* has already been mentioned. Except for the slightly larger size of the ascospores, perithecia usually possessing necks or with broken necks, as in many wood collections, *C. europhioides* might easily be mistaken for *Europhium trinacriforme*.

FIGS. 17–19. *Ceratocystis falcata*, TRTC 33037. Fig. 17. Perithecium, ×430. Fig. 18. Peridial cells, ×1700. Fig. 19. Ascospores, ×1700.

FIGS. 20–23. Fig. 20. Ascospores of *Ceratocystis minuta*, TRTC 34661, ×1700. Fig. 21. Ascospores of *Ceratocystis brunneocrinita*, TRTC 34581, ×1700. Fig. 22. Ascospores of *Ceratocystis europhioides*, TRTC 33700, ×1700. Fig. 23. Ascospores of *Ceratocystis sagmatospora*, TRTC 34600, ×1700.



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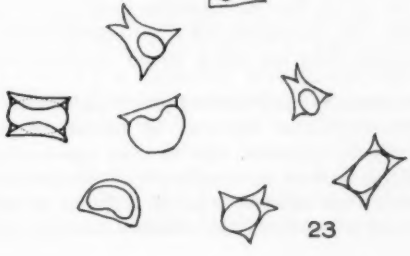

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C. europhioides is also related to *C. penicillata* (Gros.) C. Moreau. The ascospores differ in shape, those of *C. penicillata* being broadly allantoid, 6.5×2.3 μ (Grosmann 1932). The conidia also differ in shape, those of *C. penicillata* being sickle-shaped. Mathiesen-Käärik (1960) reported considerable variability in perithecial characters and in the imperfect stage of *C. penicillata*. Recently *C. penicillata* was reported from subalpine fir (Davidson 1958) but the shape of the ascospores was described as "similar to segment of an orange" and no *Leptographium* imperfect state accompanied it. This indicates the need for further taxonomic investigations of *C. penicillata*. On the basis of the distinctive ascospores, *C. europhioides* has been described as a new species.

Ceratocystis falcata Wright and Cain sp. nov. (Figs. 17–19)

Peritheciis in ligno superficialibus, basi brunneis vel nigris, 50–80 μ diam., paulo latioribus quam altis, paucis hyphis brunneis 24×2.4 μ praeditis; peridio cellularum parietibus tenuioribus ornatarum tenus 10 μ diam.; collo turbinato, brunneo vel nigro, longo 20–30 μ, basi lato 20–30 μ, ad apicem 4.5–5.2 μ et cellulis hyalinis, rotundatis, leviter prominentibus praedito. Ascis evanidis, clavatis vel lanceolatis, 20–28 × 2.4–3.2 μ. Ascosporis acicularibus a fronte conspectis, falcatis a latere conspectis, ad utrumque terminum attenuatis sed inaequalibus, septo submediano, transverso ornatis, 21–27 × 1.0–1.4 μ, emergentibus et cirrhos longos et filiformes formantibus.

Perithecia on wood, superficial, the bases brown to black, 50–80 μ in diameter, somewhat broader than high with a few brown hyphae 24×2.4 μ. Peridium of fairly thin-walled cells up to 10 μ in diameter. Neck conical, brown to black, 20–30 μ long, 20–30 μ wide at the base, 4.5–5.2 μ wide at the apex with slightly projecting, rounded hyaline cells. Asci evanescent, 8-spored, clavate to lanceolate, 20–28 × 2.4–3.2 μ. Ascospores acicular in face view, falcate in side view, attenuate at both ends but asymmetrical, with a submedian, transverse septum, 21–27 × 1.0–1.4 μ, emerging in long threadlike cirrhi.

The description of this species was made from a single collection with numerous perithecia on the sapwood. Since it was not obtained in culture, no imperfect stage can be described at this time.

Type.—On *Pinus strobus*, TRTC 33037, NW. of Nobleton, York Co., Ontario, July 1, 1957.

The small perithecia with short conical necks are quite unlike other species of the genus but do resemble young perithecia of *Ceratocystis minuta* (Siem.) Hunt. The ascospores of *C. falcata* are similar in shape to those of *C. minuta*, although they are much longer and inconspicuously septate.

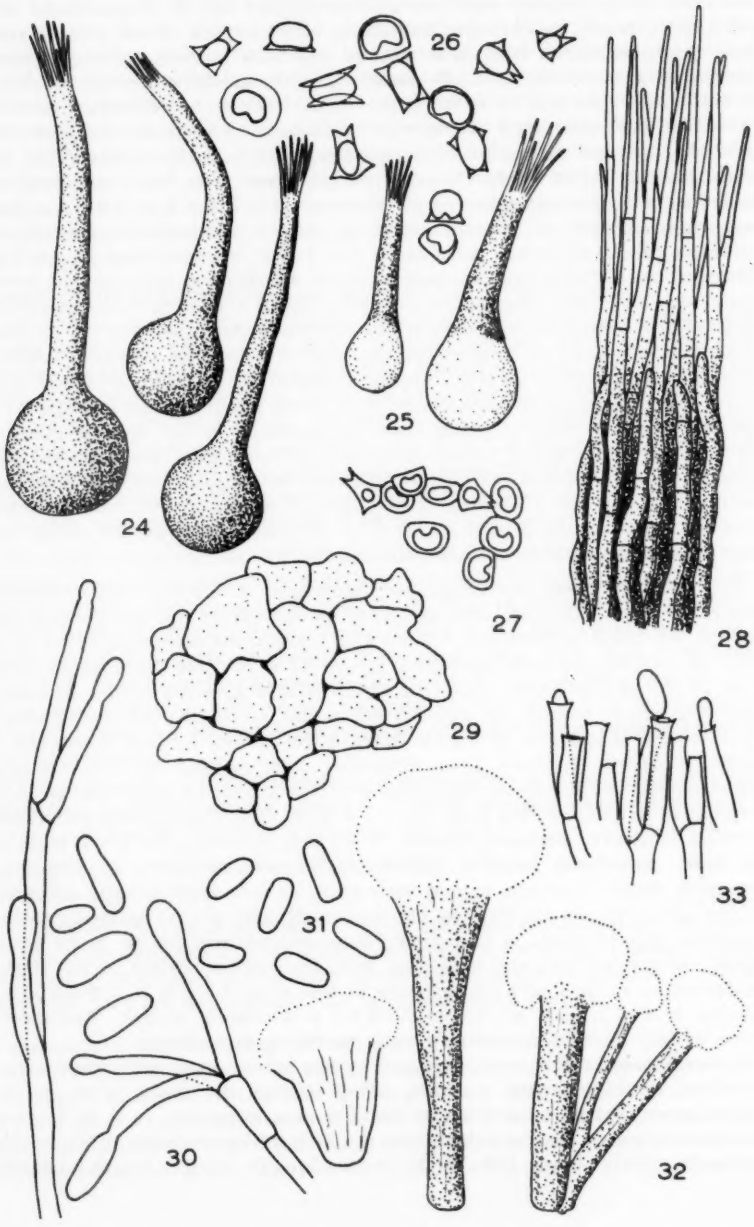
Ceratocystis sagmatospora Wright and Cain sp. nov. (Figs. 23–33)

Peritheciis in ligno et in cultura superficialibus; basibus pallido-fulvis vel nigris, globosis, non ornatis aut hyphis non disiunctis involutis, 75–160 μ diam; pariete peritheciorum ex duobus stratis cellularum composito, cellulis exterioribus intextis tenus 12 μ diam., cellulis interioribus, parietibus tenuibus ornatis, tenus 30×18 μ; collo brunneo vel nigro, ad ultimam partem pallescente,

longo 100–480 μ (hyphis ostioli comprehensis), basi lato 30–50 μ, lato 18–28 μ sub hyphis ostioli; ex fibris vel terminatis vel in hyphas ostioli prolatis composito; hyphis ostioli basi brunneis, ad ultimam partem pallido-brunneis, erectis, attenuatis, septatis, de longitudine irregularibus, longis 30–86 μ, latis 1.5–2.5 μ. Ascis novellis res densas et granulosas continentibus, clavatis, 13×4.6 μ, basi attenuatis. Ascosporis hyalinis, una cellula ornatis, late reniformibus, alte concavis a latere conspectis, ovatis a termino conspectis, late ellipsoideis a fronte conspectis, vagina gelatinosa duo labra asymmetrica formante praeditis; ascosporis (a latere conspectis) 3.6–5.8 × 2.0–3.4 μ (lato magnitudo 2.8–4.0 μ) tenus 7.3×5.8 μ (vagina comprehensa); ascosporis universis albis, aliquando succinaciis vel badiis emergentibus et cirrhum formantibus. Culturis primum pallidis et ad substratum appressis, in medio plurimum carbonem continente myceliis aeriis numerosioribus praeditis, aliis culturis postea glaucis, allis badiis. Coremiis post quattuor vel quinque dies visis, numerosis vel raris, stirpibus pallido-brunneis vel nigris, ex conidiophoribus numeri inconstantis compositis praeditis, 100–300 × 18–50 μ in agaro, 500 μ in ligno; summis coremiis ex ramis hyalinis et penicillatis compositis; ramis conidia in phialidibus terminalibus et ad apicem dilatatis efferentibus; conidiis una cellula praeditis, in massa glutinosa alba vel succinacia vel pallido-badia (ex cultura et gradu maturitatis), hyalina, obovata vel oblonga, 4.6–8.0 × 3.2–4.0 μ dispositis. Conidiis myceliorum similibus (conidiis) coremiorum, 5.8–12 × 3.2–4.6 μ. Peritheciis, plerumque numerosis, post duodecim vel quattuordecim dies maturis.

Perithecia on wood and in culture, superficial, the bases light yellowish-brown to black, globose, unornamented or enveloped by undifferentiated hyphae, 75–160 μ in diameter. Peridium of perithecium consisting of an outer layer of interlocking cells measuring up to 12 μ in diameter, and an inner layer of thin-walled cells measuring up to 30×18 μ. Neck brown to black, becoming lighter toward the tip, 100–480 μ long (including ostiolar hyphae), 30–50 μ wide at base, 18–28 μ wide beneath the ostiolar hyphae, composed of filaments either terminating or extending into ostiolar hyphae. Ostiolar hyphae brown at the base, light brown at the tip, erect, tapering, septate, irregular in length, 30–86 μ long, 1.5–2.5 μ wide. Asci when young with dense granular contents, 8-spored, clavate, 13×4.6 μ, narrowing to 1.6 μ wide at the base. Ascospores hyaline, unicellular, broadly reniform, in side view appearing deeply concave, in end view oval, in face view broadly ellipsoid, having gelatinous sheath forming two asymmetrically placed brims appearing cucullate in side view, in end view brims approaching at an acute angle below concave surface, and spreading from the convex surface of the spore, quadrangular in face view; dimensions in side view 3.6–5.8 × 2.0–3.4 μ (in extreme width 2.8–4.0 μ) up to 7.3×5.8 μ including sheath. Ascospores white, amber, or reddish-brown in mass, emerging in a cirrhus.

Cultures pale at first and appressed to the substratum, with more aerial mycelium on high-carbon medium, some isolates darkening with age to grayish-green, others to reddish-brown. Coremia appearing in 4 to 5 days, abundant or scarce, having light brown to black stalks consisting of a variable number of conidiophores, 100–300 × 18–50 μ in agar, 500 μ in length on wood;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the heads consisting of hyaline, penicillate branches producing conidia on terminal phialides with flaring collarette; conidia collecting in a viscid head varying in color in various isolates and with age from white to amber to pale reddish-brown, unicellular, hyaline, obovate to oblong, narrower toward the end of attachment, 4.6–8.0 × 3.2–4.0 μ. Mycelial conidia formed as on coremia at the ends of hyaline hyphae showing all transitional stages of branching to that of coremia, similar to conidia of coremia, 5.8–12 × 3.2–4.6 μ. Perithecia usually abundant, maturing in 12 to 14 days.

The species was found fruiting in bark beetle galleries, on the sapwood and on freshly cut surfaces of *Picea mariana*, *Pinus resinosa*, and *Pinus strobus*. Only a slight grayish-green discoloration was observed.

Type.—On *Picea mariana*, TRTC 36427, Twp. West of 11H, Challener Lake, Sudbury Dist., Ontario, June 20, 1960.

Collections.—Ontario: on *Pinus resinosa*, TRTC 34600, NE. of Mansfield, Dufferin Co., Nov. 8, 1958; on *Pinus strobus*, TRTC 33034, NW. of Nobleton, York Co., July 1, 1957; on *Pinus strobus*, TRTC 36245, 36251, 36255, 36264, 36265, Twp. West of 11H, Challener Lake, Sudbury Dist., June 20, 1960; on *Pinus strobus*, TRTC 36246, Twp. 5F, Aubinadong R., Algoma Dist., June 17, 1960; on *Picea mariana*, TRTC 36263, Twp. West of 11H, Challener Lake, Sudbury Dist., June 20, 1960.

C. sagmatospora is very similar to *C. olivacea* and *C. obscura* in both perithecial morphology and imperfect stage. The separation is based largely on the ascospores, which differ in shape (culture SK-11-8-52, Mathiesen-Käärik) from those of *C. olivacea* (Mathiesen 1951) and are larger than those of *C. obscura* (Davidson 1935, Fig. 1J; Hunt 1956, Plate 1, Fig. 2). The ostiolar hyphae attain the length of those in *C. olivacea*. Shorter ostiolar hyphae as described for *C. obscura* were encountered on young perithecia. Since the ostiolar hyphae are erect extensions of neck filaments and since they elongate apically, measurement of their length depends not only on the location of their origin but also on the age of the perithecium. Conidia produced on coremia are somewhat smaller in *C. olivacea* than in *C. sagmatospora* and *C. obscura*. Perithecia develop more rapidly than in either *C. olivacea* or *C. obscura*. *C. vesca* (Davidson 1958) may also be a related species, differing in the smaller perithecia, ascospores, and conidia.

Acknowledgments

Grateful acknowledgment is made to Dr. Aino Mathiesen-Käärik for cultures of species from Sweden. The writers are also indebted to Professor Eric Jorgensen, Dr. James Reid, and Dr. E. Robena Luck-Allen for their interest in the problem, and to Mrs. Paul Hiscox for the Latin descriptions.

FIGS. 24–33. *Ceratocystis sagmatospora*. Fig. 24. Perithecia in culture, TRTC 34600, ×120. Fig. 25. Perithecia with light bases, TRTC 34600, ×120. Fig. 26. Ascospores in water, TRTC 33034, ×1200. Fig. 27. Ascospores in lactophenol, TRTC 33034, ×1200. Fig. 28. Ostiolar hyphae from a culture, TRTC 34600, ×750. Fig. 29. Peridial cells from a culture, TRTC 34600, ×1200. Fig. 30. Conidial production in a young culture, TRTC 34600, ×1200. Fig. 31. Conidial production on *Graphium* conidiophore, TRTC 33034, ×1200. Fig. 32. *Graphium* conidiophores, TRTC 33034, ×120. Fig. 33. Phialides at tip of conidiophore, TRTC 33034, ×1200.

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References

ANDRUS, C. F. 1936. Cell relations in the perithecium of *Ceratostomella multiannulata*. Mycologia, **28**, 133–153.

ANDRUS, C. F. and HARTER, L. L. 1933. Morphology of reproduction in *Ceratostomella fimbriata*. J. Agr. Research, **46**, 1059–1078.

——— 1937. Organization of the unwalled ascus in two species of *Ceratostomella*. J. Agr. Research, **54**, 19–46.

BAKSHI, B. K. 1951. Development of perithecia and reproductive structures in two species of *Ceratocystis*. Ann. Botany, n.s., **15**, 53–61.

DAVIDSON, R. W. 1935. Fungi causing stain in logs and lumber in the Southern States, including five new species. J. Agr. Research, **50**, 789–807.

——— 1958. Additional species of Ophiostomataceae from Colorado. Mycologia, **50**, 661–670.

ELLIOTT, J. A. 1925. A cytological study of *Ceratostomella fimbriata* (E. & H.) Elliott. Phytopathology, **15**, 417–422.

GROSMANN, H. 1932. Über die systematischen Beziehungen der Gattung *Leptographium* Lagerberg et Melin zur Gattung *Ceratostomella* Sacc. Hedwigia, **72**, 183–194.

GWYNNE-VAUGHAN, H. C. I. and BROADHEAD, Q. E. 1936. Contributions to the study of *Ceratostomella fimbriata*. Ann. Botany, **50**, 747–758.

HALSTED, B. D. and FAIRCHILD, D. G. 1891. Sweet-potato black rot. J. Mycol. **7**, 1–11.

HUNT, JOHN. 1956. Taxonomy of the genus *Ceratocystis*. Lloydia, **19**, 1–58.

HUTCHINSON, S. A. 1950. The perithecia of *Ophiostoma majus* (Van Beyma) Goidànich. Ann. Botany, n.s., **14**, 115–125.

LAGERBERG, T., LUNDBERG, G., and MELIN, E. 1927. Biological and practical researches into blueing in pine and spruce. Svensk Skogsvårdsf. Tidskr. **25**, 145–272.

LUTTRELL, E. S. 1951. Taxonomy of the Pyrenomycetes. Univ. Missouri Studies, **24**(3), 1–120.

MATHIESEN, A. 1951. Einige neue *Ophiostoma*-arten in Schweden. Svensk Botan. Tidskr. **45**, 203–232.

MATHIESEN-KÄÄRIK, A. 1953. Eine übersicht über die gewöhnlichsten mit Borkenkäfern assozierten Bläuepilze in Schweden und einige für Schweden neue Bläuepilze. Medd. Statens Skogsforskningsint. Sweden, **43**(4), 1–74.

——— 1960. Studies on the ecology, taxonomy and physiology of Swedish insect-associated blue stain fungi, especially the genus *Ceratocystis*. Oikos, **11**, 1–25.

MELIN, E. and NANNFELDT, J. A. 1934. Researches into the blueing of ground wood-pulp. Svensk Skogsvårdst. Tidskr. **32**, 397–616.

MITTMAN, G. 1932. Kulturversuche mit Einsporstämmen und zytologische Untersuchungen in der Gattung *Ceratostomella*. Jahrb. wiss. Botan. **77**, 185-219.

MOREAU, M. and MOREAU, V. 1952. Sur le développement du *Ceratocystis moniliformis* (Hedgcock) nov. comb. Rev. mycol. **17**, 141–153.

MÜNCH, E. 1907. Die Blaufäule des Nadelholzes. Naturw. Z. Forst-u. Landwirtsch. **5**, 531–573.

NANNFELDT, J. A. 1932. Studien über die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. Nova Acta Regiae Soc. Sci. Upsaliensis, Ser. IV, **8**(2), 1–368.

PARKER, A. K. 1957. *Europhium*, a new genus of the Ascomycetes with a *Leptographium* imperfect state. Can. J. Botany, **35**, 173–179.

ROBAK, H. I. 1932. Investigations regarding fungi on Norwegian ground wood pulp and fungal infection at Wood Pulp Mills. Nyt. Mag. Naturvidensk. **71**, 185–330.

SARTORIS, G. B. 1927. A cytological study of *Ceratostomella adiposum* (Butl.) comb. nov., the black-rot fungus of sugar cane. J. Agr. Research, **35**, 577–585.

TAYLOR-VINJE, M. 1940. Studies in *Ceratostomella montium*. Mycologia, **32**, 760–775.

VARITCHAK, B. 1931. Contributions à l'étude du développement des Ascomycetes. Le Botaniste, **23**, 1–184.

STUDIES OF SOIL FUNGI

III. NEW SPECIES OF CONIOCHAETA, CHAETOMIDIUM, AND THIELAVIA[1]

ROY F. CAIN

Abstract

Descriptions and illustrations are given for three new species of Ascomycetes having colored, one-celled ascospores and isolated from forest soil by J. H. Warcup. *Coniochaeta tetraspora* Cain sp. nov. has dark spiny hairs on the perithecium. The phialospores and blastospores are typical of the genus. The soil from which it was isolated came from Queensland, Australia. *Chaetomidium minutum* Cain sp. nov. has short, curved hairs on the globose, nonostiolate perithecia. The soil sample came from N. Queensland, Australia. *Thielavia variospora* Cain sp. nov. has black, nonostiolate, bare perithecia and was isolated from soil collected in New Guinea.

Introduction

Dr. J. H. Warcup obtained a number of interesting species of Ascomycetes in culture from forest soils obtained in New Guinea and Australia. Three of these have dark-colored, one-celled ascospores and belong in three separate genera, *Coniochaeta*, *Chaetomidium*, and *Thielavia*. The first has black, ostiolate perithecia covered with short, dark, spiny hairs. The last two have globose, nonostiolate perithecia. The species of *Chaetomidium* has short, curved hairs on the perithecium. That of *Thielavia* has bare perithecia.

Dried specimens have been deposited in the Cryptogamic Herbarium, University of Toronto (TRTC).

Coniochaeta

Coniochaeta tetraspora Cain sp. nov. (Figs. 1–17)

Colony (on Leonian + yeast extract) thin, white at first, becoming pinkish as conidia develop, smooth, slimy, glistening, without aerial hyphae, forming slimy mass of conidia. Hyphae hyaline, with septa spaced fairly closely, vacuolate, 3–4 μ in diameter. Phialides abundant, variable in size and shape, usually reduced to mere openings in cells of hyphae, usually not distinctly differentiated morphologically but producing conidia in succession at aperture. Conidia one-celled, hyaline, ellipsoid to nearly oblong, variable in size and shape, 4–11 $\times$ 1.0–4.0 μ, thin-walled, sometimes producing blastospores by budding, light pinkish in slimy mass. Perithecia developing in abundance in central part of colony, densely aggregated in some areas, scattered separately in others, superficial, pyriform, ostiolate at apex but without distinct neck region, 135–220$\times$80–190 μ, black, upper part hairy. Peridium of perithecium fairly thin, membranaceous but opaque. Cells of peridium dark brownish-black, obscure. Hairs spiny, short, straight, rigid, dark brown, 15–20 $\times$ 2.5–3.5 μ (width measured at base). Asci four-spored (at maturity) (eight-

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spored when ascospores first delimited), cylindrical, 50–55 × 8–11 μ, broadly rounded above, with very slight or no indication of thickened ring in apex, slightly attenuated below into very short, stout stipe, with indistinct crozier at base. Paraphyses filiform, short, septate, scanty, 2.5–4.0 μ in diameter, becoming swollen in age. Periphyses forming a distinct layer but short. Ascospores obliquely, uniseriate, compressed on two sides, narrowly ellipsoid in one view, broadly ellipsoid in other view, 10–15 × 6.5–8.0 × 5.0–6.0 μ, hyaline at first, becoming olivaceous-brown and finally dark brown and opaque, one-celled. Germinal slit extending entire length of ascospore along narrower face.

Colonia (in agaro Leonian + yeast extract) tenui, primum alba, deinde rosea (conidiis crescentibus), levi, limosa, nitenti, sine hyphis aeriis, massam limosam conidiorum formante. Hyphis hyalinis, septis aliguantulum densis ornatis, spatiis vacuis praeditis, 3–4 μ diam. Phialidibus numerosis, amplitudine et forma variis, ad ipsa foramina in cellulis hypharum plerumque redactis, plerumque sine forma conspicue distincta sed conidia ex ordine ad aperturam effundentibus. Conidiis una cellula praeditis, hyalinis, ellipsoideis vel paene oblongis, amplitudine et forma variis, 4–11 × 1.0–4.0 μ, parietibus tenuibus ornatis, aliquando gemmando blastosporas effundentibus, in massa limosa pallido-roseis. Peritheciis in parte media coloniae abundanter effusis, in aliis partibus dense congregatis, in aliis partibus separatim sparsis, superficialibus, piriformibus, in apice ostiolatis sed sine parte collari distincta, 135–220 × 80–190 μ, nigris, parte superiore pilosa ornatis. Peridio perithecii aliquantulum tenui, membranaceo sed haud translucido. Cellulis peridii valde brunneo-nigris, indistinctis. Pilis similibus spinae, brevibus, rectis, rigidis, atro-brunneis, basi latis 15–20×2.5–3.5 μ. Ascis quattuor sporis ornatis maturis, octosporis (ascosporis primum formatis), cylindraceis, 50–55 × 8–11 μ, superne late rotundatis, annulo incrassato in apice sito levissime spectabili ornatis vel sine annulo, inferne in stipitem brevissimum, crassum leviter attenuatis, basi croca indistincta praeditis. Paraphysibus filiformibus, brevibus, septatis, paucis, 2.5–4.0 μ diam., turgidis senescentibus. Periphysibus stratum distinctum formantibus sed brevibus. Ascosporis oblique uniseriatis, a duobus lateribus compressis, ab altero latere anguste ellipsoideis, ab altero latere late ellipsoideis conspectis, 10–15 × 6.5–8.0 × 5.0–6.0 μ, primum hyalinis, deinde olivaceo-brunneis et postremo atro-brunneis et haud translucidis, una cellula ornatis. Hilo germinali in totam longitudinem ascosporae secundum superficiem angustiorem extenso.

Isolated in pure culture from forest soil, Binna Burra, Queensland, Australia, J. H. Warcup A 23/4. TRTC 36859 **type**.

This species can be distinguished from *Sordaria rabenhorstii* Niessl by means of the dark spiny hairs on the perithecium, the shorter stipe on the ascus, the smaller ascospores which are compressed from two sides instead of being ellipsoid, and the elongated germinal slit.

The conidia are produced in abundance on phialides, which are very indistinct, often mere openings in cells in the hyphae. The phialospores continue to produce blastospores by budding. The two kinds of conidia are indistinguishable

in form and color and accumulate to form a pink, slimy layer on the surface of the agar. In this respect *C. tetraspora* resembles many other species of *Coniochaeta* grown in culture. As in cultures of other species of the genus, perithecia are not produced on all media tried and no discharge of ascospores has been observed. In addition, cultures tend to lose their ability to produce perithecia after several transfers. The perithecia have a definite ostiole but the asci tend to break at the opening without actually discharging the ascospores into the air. Thus there is an accumulation of ascospores at the apex of the perithecium.

When the ascospores are first delimited there are eight in each ascus. They are small and hyaline. Each is enclosed in a large vacuole. However, four of these rapidly disappear and the other four begin at once to enlarge. It is necessary to observe very young stages in order to see the eight ascospores.

The genus *Coniochaeta* occupies a very isolated position in the Ascomycetes. In its conidial stage as well as in most of its other characteristics it is completely different from the genera of the Xylariaceae. The two appear to have only one structure in common, the elongated germinal slit in the ascospore. This important feature sets *Coniochaeta* apart from other genera with dark one-celled ascospores such as *Sordaria*, *Podospora*, *Bombardia*, *Gelasinospora*, and *Melanospora*.

Chaetomidium

Chaetomidium minutum Cain sp. nov. (Figs. 18–23)

Colonies white, thin, cottony, spreading rapidly, with indefinite margin. Perithecia globose, superficial, black, hairy, 40–60 μ in diam., produced abundantly in a thin, loose weft of white aerial mycelium. Peridium of perithecium very thin, membranaceous, brown. Cells of peridium angular, 5–8 μ in diameter, brown, nearly opaque, moderately thin-walled. Hairs 10–25 μ in length, 3 μ wide at base, tapering gradually from base to blunt apex, brown, minutely roughened, 2–4 septate, curved, forming fairly dense layer over entire perithecium. Asci 8-spored, subglobose, 14–18$\times$13–15 μ, irregularly disposed in perithecium, evanescent, no visible apical structure. No paraphyses. Ascospores ovate, 6.5–8.0$\times$5.0–5.5 μ, slightly pointed at one end, one-celled, olivaceous-brown. Germinal pore circular, 1 μ in diameter, single in ascospore, at pointed end.

Coloniis albis, tenuibus, gossypio similibus, celeriter crescentibus, margine indefinito ornatis. Peritheciis globosis, superficialibus, nigris, pilosis, 40–60 μ diam., in strato tenui raro mycelii albi aerii abundanter effusis. Peridio perithecii tenuissimo, membranaceo, brunneo. Cellulis peridii angulatis, 5–8 μ diam., brunneis, haud translucidis, parietibus modice tenuibus ornatis. Pilis longis 10–25 μ, basi latis 3 μ, a basi ad apicem hebetem paulatim attenuatis, brunneis, subtiliter asperatis, 2–4 septis praeditis, curvatis, in strato densiore per totum perithecium dispositis. Ascis octosporis, subglobosis, 14–18$\times$13–15 μ, in perithecio irregulariter dispositis, evanidis, sine apice spectabili. Sine paraphysibus. Ascosporis ovatis, 6.5–8.0 $\times$ 5.0–5.5 μ, a termino leviter attenuatis, una cellula praeditis, olivaceo-brunneis. Foramine germinali rotundo, 1 μ diam., singulo in ascosporo, in termino attenuato sito.

Isolated in pure culture from forest soil, N. Queensland, Australia, J. H. Warcup L 25/1/1. TRTC 36862 **type**.

This species is distinguished by the minute perithecia, the short, curved, septate hairs and small, ovate ascospores.

Chaetomidium is separated from *Thielavia* by the hairs on the perithecia. It may be included in the family Tripterosporaceae if the absence of an ostiole is to be regarded as of sufficient importance for family separation. The genus is close to *Chaetomium* from which it may have had its origin. It might therefore be preferable to include it in the family Chaetomiaceae.

Thielavia

Thielavia variospora Cain sp. nov. (Figs. 24–31)

Colony spreading rapidly, with very scanty, white, aerial mycelium. Perithecia globose, superficial, scattered or heaped in clusters, black, smooth, nonostiolate, 125–200 μ in diameter. Peridium light brown, semitransparent, thin, coriaceous. Cells of peridium very distinct in outline, with very thin walls, light brown, irregular in size and shape, 3–8 μ in diameter. Asci 8-spored, subglobose to ellipsoid, irregularly disposed, evanescent, when immature hyaline, filled with minute globules, measuring 20×15 μ, just prior to ascospore formation with 8 globules, when mature measuring 30–35×18–20 μ, very broadly rounded above, tapering slightly toward broad base, nondehiscent, with no apical structure visible, evanescent. No paraphyses visible. Ascospores variable in shape, ellipsoid, somewhat triangular, or irregularly angular reniform, frequently with one or more flattened sides, rarely concave on one side, 9–15×5.5–8.0 μ, one-celled, becoming distinctly olivaceous and finally olivaceous-brown. Germinal pore single in each ascospore, circular, 1 μ in diam., usually located at one end, rarely lateral.

Colonia celeriter crescente, mycelio valde exiguo, albo, aerio praedita. Peritheciis globosis, superficialibus, sparsis vel in uvas coacervatis, nigris, levibus, non ostiolatis, 125–200 μ diam. Peridio pallido-brunneo, semitranslucido, tenui, coriaceo. Cellulis peridii valde distinctis, tenuissimis parietibus ornatis, pallido-brunneis, irregularibus, 3–8 μ diam. Ascis octosporis, subglobosis vel ellipsoideis, irregulariter dispositis, evanidis, immaturis hyalinis, globulis minimis impletis, 20×15 μ, octoguttulatis ante ascosporos factos, maturis 30–35 × 18–20 μ, superne latissime rotundatis, ad basem latum leviter attenuatis, non dehiscentibus, sine apice spectabili, evanidis. Nullis paraphysibus spectabilibus. Ascosporis formis variis ornatis, ellipsoideis, leviter triangularibus, vel irregulariter angulatis, reniformibus, saepe uno vel pluribus lateribus complanatis ornatis, raro ab uno latere concavis, 9–15× 5.5–8.0 μ, una cellula ornatis, deinde distincte olivaceis et postremo olivaceo-brunneis. Foramine germinali singulo in quoque ascosporo, rotundo, 1 μ diam., plerumque ab uno termino sito, raro a latere.

Isolated in pure culture from forest soil, near Madang, New Guinea, J. H. Warcup A 2/1. TRTC 36863 **type**.

This species is easily distinguished by means of the irregular shape of the ascospores.

The genus *Thielavia* may be classified in the family Tripterosporaceae for the present. It may have had its origin from *Sordaria*, *Podospora*, or a related genus in a line or lines not directly through *Tripterospora*.

Acknowledgments

I am deeply indebted to Dr. J. H. Warcup, who kindly furnished cultures of the fungi described above.

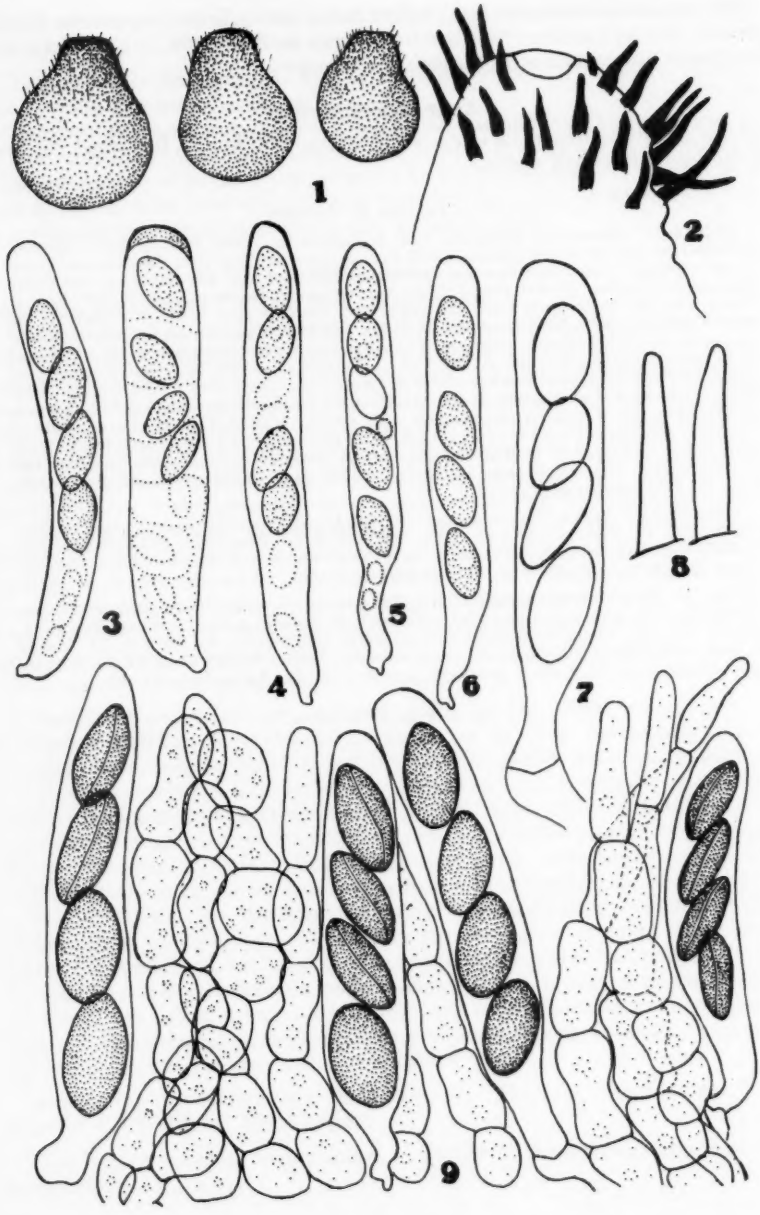
Explanation of Figures

Figs. 1–17. *Coniochaeta tetraspora*. Fig. 1. Perithecia, ×100. Fig. 2. Neck region of perithecium with hairs and ostiole, ×400. Fig. 3. Two asci just after the circumscription of the ascospore; the lower four ascospores are indistinct and disintegrating, the upper four are still hyaline; ×1200. Fig. 4. The aborting ascospores are in pairs, ×1200. Fig. 5. Ascus with the third spore from the upper end aborting after some enlargement; the other three ascospores have nearly disappeared; four have contents but are still immature and hyaline; ×1200. Fig. 6. Ascus with four hyaline immature ascospores; the other four have aborted and disappeared; ×1200. Fig. 7. Mature ascus with four mature ascospores, ×1200. Fig. 8. Outline of two hairs from upper part of perithecium, ×1200. Fig. 9. Four mature asci with hyaline paraphyses, ×1200. Fig. 10. Conidium forming blastospores, ×1200. Fig. 11. Conidium germinating with hypha, ×1200. Fig. 12. Later stage of conidium germinating with hypha, ×1200. Fig. 13. Septate and branching hypha from germinating conidium in 24-hour culture, ×1200. Fig. 14. Hyphae with phialides and conidia, ×1200. Fig. 15. Mature ascospores, ×1200. Fig. 16. Mature ascospores, ×800. Fig. 17. Four mature asci, ×800.

Figs. 18–23. *Chaetomidium minutum*. Fig. 18. Group of perithecia on surface of agar, ×200. Fig. 19. Perithecium with hairs, ×800. Fig. 20. Four hairs from perithecium, ×1200. Fig. 21. Perithecium showing cells of peridium and hairs, ×1200. Fig. 22. Three mature asci, ×1200. Fig. 23. Ascospores. ×1200.

Figs. 24–31. *Thielavia variospora*. Fig. 24. Group of perithecia on surface of agar, ×100. Fig. 25. Two young asci filled with small oil globules, before ascospore formation, ×1200. Fig. 26. Young ascus just before ascospore delimitation, ×1200. Fig. 27. Young hyaline ascus with eight oil globules, ascospores not visible, ×1200. Figs. 28, 29. Mature asci, ×1200. Fig. 30. Cells of peridium, ×1200. Fig. 31. Ascospores, ×1200.

Note: Figs. 1–31 follow.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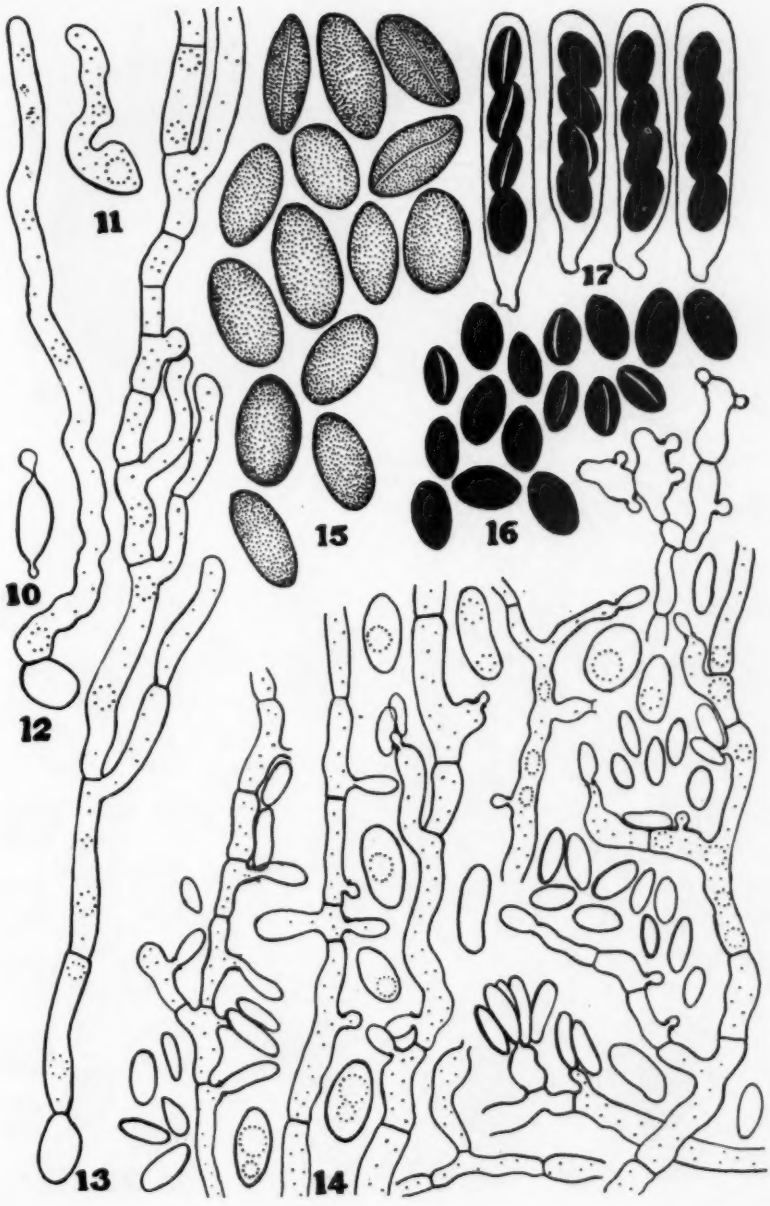
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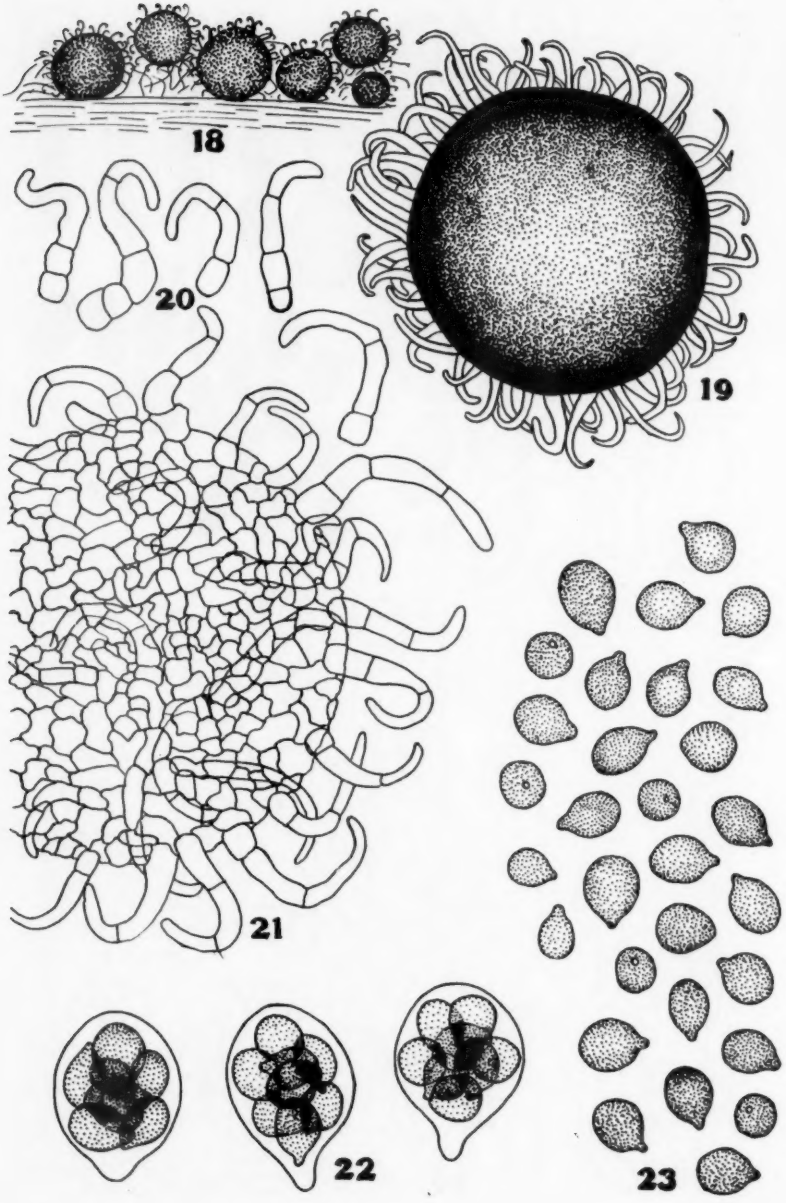
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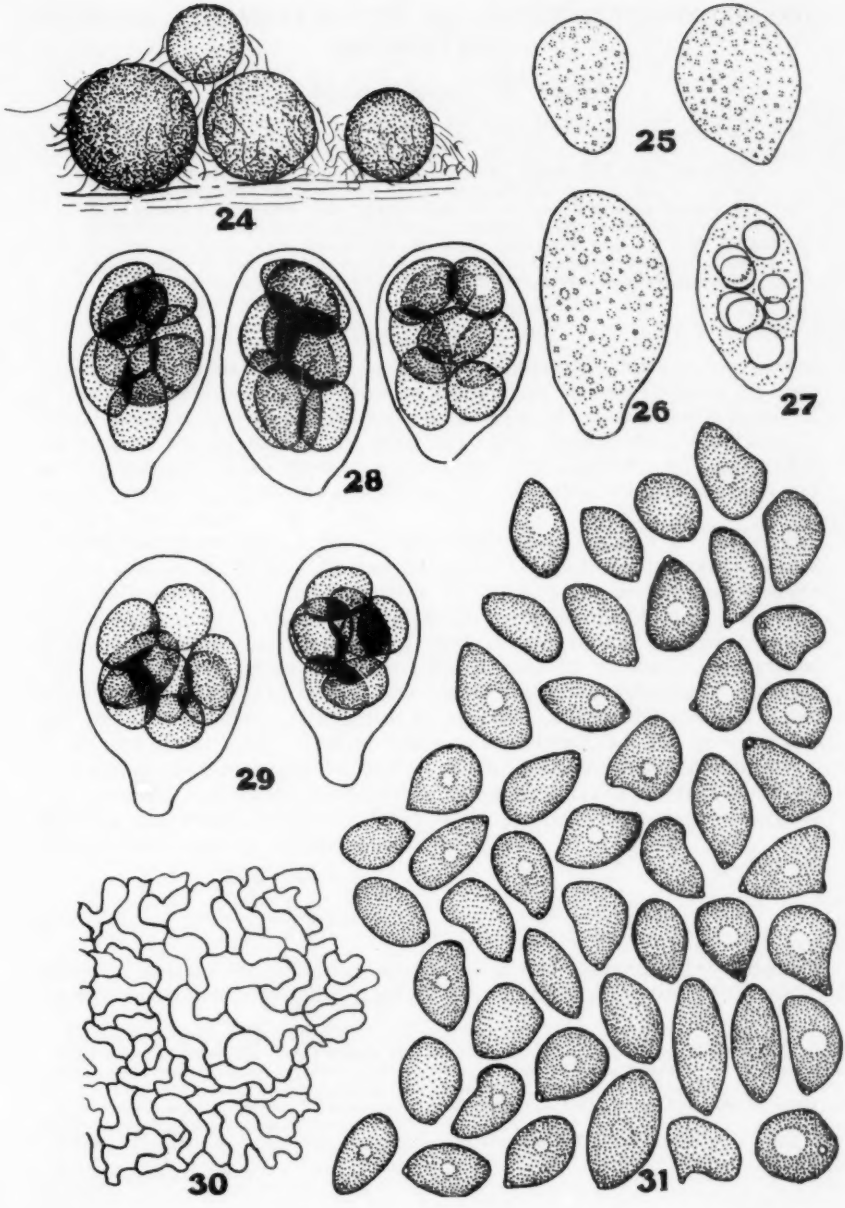
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MODIFIED INCOMPATIBILITY OF BUCKWHEAT FOLLOWING IRRADIATION[1]

K. D. SHARMA[2] AND J. W. BOYES[3]

Abstract

Among plants grown from buckwheat "seeds" which had been exposed to X rays or thermal neutrons two thrum-flowered plants each had a branch bearing modified flowers. One thrum plant had a branch bearing homostyled flowers and the other a branch bearing pin flowers. A thrum plant in the progeny of the latter plant was self-fertile, produced no progeny in crosses with other thrum plants, but was fertile in crosses with pin-flowered plants. These cases are discussed in relation to a postulated S supergene controlling incompatibility in buckwheat.

Introduction

Certain buckwheat plants (*Fagopyrum esculentum*) produce long-styled flowers (pin) whereas others produce short-styled flowers (thrum). In such cases legitimate crosses involve the transference of pollen from stamens of one type to the stigmas of the other type and illegitimate crosses involve such transfers between flowers of the same type (Darwin, 4). Seed yields are high following legitimate crosses but very low after illegitimate ones and selfing in buckwheat.

A number of workers, including Althausen (1), Dahlgren (3), Eghis (6), Garber and Quisenberry (10), and Saknarov (18), have presented evidence that the inheritance of style length in this species is controlled by a single gene difference, the gene for short style (S) being dominant to that for long style (s). The situation is, however, complicated by the fact that short-styled flowers also differ from long-styled ones in having long stamens, larger pollen grains (Tatebe, 19), the thrum stylar incompatibility reaction, and the thrum pollen incompatibility reaction. Since Dowrick (5), Ernst (7, 8), and others have presented evidence in *Primula* that the gene for this complex of characters can be broken down, apparently by crossing over, into subunits, the S gene is now regarded as a supergene in that genus by Dowrick and others. Following Dowrick we postulate that the S supergene of buckwheat may be organized as follows:

$$\frac{\text{Thrum Ss}}{\text{G } I^S \; I^P \; P \; A \;/\; g \; i^S \; i^P \; p \; a} \qquad \frac{\text{Pin ss}}{g \; i^S \; i^P \; p \; a \;/\; g \; i^S \; i^P \; p \; a}$$

Here we assume the following control of flower characters by subgenes: G, thrum short style length; I^S, thrum incompatibility reaction of the style;

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Contribution from the Department of Genetics, McGill University, Montreal, Que., Canada. This report is based on part of a thesis submitted to the Department by K. D. Sharma in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
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I^P, thrum incompatibility reaction of the pollen; P, thrum large pollen size; and A, thrum greater anther height.

Materials and Methods

Thirteen samples of 100 newly harvested fruits ("seeds") of diploid Silverhull Buckwheat were sent to the Brookhaven National Laboratory in September, 1957, for irradiation. Moisture content was stabilized at 60% relative humidity; six separate samples were exposed to X-ray doses of 5-, 10-, 20-, 25-, and 30-thousand r, respectively; six other separate samples were exposed for 5, 10, 15, 20, 25, and 33.5 hours at a flux value of 4.85×10^8 thermal neutrons/cm^2/sec, respectively; and one sample was kept as an untreated control. Fifty seeds from each of these 13 samples were planted in the greenhouse in November 1957 and a single plant, from seed of the sample exposed to 20,000 r, had a branch bearing flowers with long stamens and long styles (i.e. homostyled) and other branches with apparently normal thrum flowers. This was one plant out of the 600 treated with X rays and it will be referred to as plant A.

Five samples of 200 seeds each, from the same original stock, were treated under the same conditions at Brookhaven in September, 1958; one sample received 10,000 r and another 15,000 r; another sample received 4 hours at a flux value of 4.25×10^8 thermal neutrons/cm^2/sec and still another 8 hours at the same flux value; and a sample was kept as an untreated control. Two lots of 50 seeds from each sample were planted in October 1958. In the total of 400 plants grown from these treated seeds one plant (B) was found, in the 4-hour thermal neutron treatment group, that bore six pin flowers, six thrum, and a half-pin half-thrum flower on one branch and 38 thrum flowers on other branches. These flowers were used in various crosses to produce seeds of the three following categories: (*a*) nine seeds from crosses involving plant B as ♀ or both ♀ and ♂ parents, (*b*) eight seeds from crosses involving plant B as ♂ (pin) parent, and (*c*) eight seeds from crosses involving plant B as ♂ (thrum) parent. These were planted with eight seeds from nonirradiated plants as a control group. All of the plants from these seeds were selfed on 12 occasions in February and March, 1959. All three categories mentioned above were combined into a single "treated" group for comparison with the control group in seed production.

Observations

Plant A produced homostyled flowers on one branch (long stamens, long styles, see Fig. 3) and on other branches 21 thrum flowers, all of which were selfed in December, 1957, and January, 1958, but no seeds whatever were formed. In February four other thrum flowers and three other homostyled flowers on this plant were crossed using a control pin plant as ♂ parent. Two seeds started to develop on thrum flowers and one on one homostyled flower, before the plant was accidentally seriously damaged and died. These results suggest that the incompatibility reactions of pollen and style in the homostyled flowers were the same as those of the thrum flowers on the same plant. Also anther heights and pollen diameters for both types of flowers were the same as in normal thrum flowers of control plants. Apparently this plant was

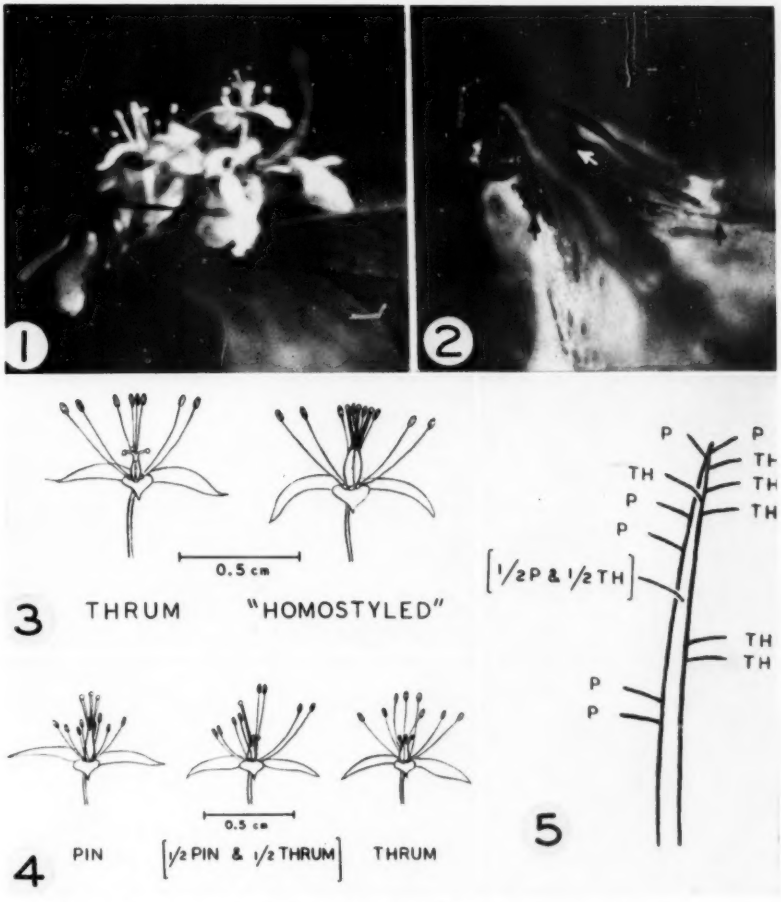

FIG. 1. Branch of plant B, bearing both pin (left) and thrum (right) flowers.
FIG. 2. Terminal part of "seed" from half-pin half-thrum flower on plant B: note two short styles and one long.
FIG. 3. Drawings of thrum and "homostyled" flowers of Plant A.
FIG. 4. Drawings of pin, half-pin half-thrum, and thrum flowers of plant B.
FIG. 5. Sketch of the arrangement of pin, half-pin half-thrum, and thrum flowers on a branch of plant B.

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a genetic chimaera in which a part of the S supergene was modified in one sector.

Plant B had a branch which produced six pin and six thrum flowers together with a flower which was half-pin, with four short stamens and one long style, and half-thrum, with four long stamens and two short styles (see Figs. 1, 2, 4). These flowers were arranged on the branch as shown in Fig. 5 and three other branches bore 38 normal thrum flowers. Anther heights and pollen diameters in both pin and thrum flowers on this plant were within the size ranges for pin and thrum flowers, respectively, on control plants. This was also true for the pin and thrum parts of the exceptional flower. Also the differences in stylar lengths in the three types of flowers were very conspicuous (Fig. 4).

Flowers of plant B were used for legitimate crosses between flowers on the same plant and for legitimate crosses with two nonirradiated control plants. One of the control plants (C1) had pin flowers, the other (C2) had thrum flowers, and 20 selfed flowers on each produced no seed at all. Selfing thrum flowers on plant B also gave no seed. Results of these crosses, expressed as number of seed set over the number of flowers pollinated, are shown below:

B Pin × B Thrum = 4/6
B Thrum × B Pin = 2/3
B Thrum selfed = 0+1 empty/5
B 1/2 Pin × 1/2 Thrum = 1/1
B Thrum × C1 Pin = 2/5
B Thrum × C2 Thrum = 0/4
C1 Pin × B Thrum = 13/27
C2 Thrum × B Thrum = 0/4
C2 Thrum × B Pin = 8/11

These results clearly indicate that the incompatibility reactions of the pollen and style in the pin and thrum flowers (and the parts of the one flower) on plant B were as expected in pin and thrum flowers, respectively, on normal plants. Apparently plant B was a chimaera in which some of the flowers had modified morphology, incompatibility reactions, and pollen diameter.

As mentioned above, the various progenies from the above crosses involving plant B were grown and selfed at the same time as control plants. On treated plants 16 seeds were obtained from 555 selfed flowers (i.e. 2.88%) whereas control plants gave 0 for 71 selfed flowers (0.00%). Treated pin plants gave 1 seed for 253 flowers (i.e. 0.40%) whereas control pin plants produced no seeds on 52 flowers. On the other hand, treated thrum plants produced 15 seeds on 302 selfed flowers (i.e. 4.93%) whereas none were produced on 32 control thrum flowers. The difference between pin and thrum treated plants is highiy significant. One of the treated thrum plants was, however, highly self-fertile producing 12 full seeds (and 7 empty ones) on 42 selfed flowers (28.6%). This plant (T) was crossed to two nonirradiated control plants (C3 and C4) with the following results expressed as seeds per flower pollinated: T Thrum × C3 Thrum = 0/26; C3 Thrum × T Thrum = 0/20; T Thrum × C4 Pin = 9/10. It would seem that the self-fertility of plant T is not a consequence of a breakdown of its incompatibility system, as the pollen and stylar reactions with other plants were not changed.

Discussion

The observations on plants A and B are of interest in relation to the genetic nature of the S locus. The following considerations lead to the suggestion that the S locus of buckwheat may be organized as a supergene similar to

that suggested by Dowrick for *Primula* and as postulated for buckwheat in the introduction to this report.

The homostyled flowers of plant A apparently differed from thrum flowers on the same plant only in the length of the style. Also they were borne on one branch only, with thrum flowers on the other branches. Hence it seems very likely, though not certain, that the genotype of the X-ray-treated embryo was Ss. If this was so, then the sector-bearing homostyled flowers may have arisen through mutation or conversion of S, through the occurrence of a small deletion, or through somatic crossing-over within the S locus. A mutation or conversion of a dominant subgene G→g could explain the origin of the homostyled flowers. A very small deletion of part of the S locus could operate in the same way. Somatic crossing-over within the S supergene is another possibility providing the G subgene is at one end or the other of the S supergene. All three interpretations involve a need to regard the S locus as a supergene.

Somewhat similar considerations apply to the presence of part of a branch bearing pin (or half-pin) flowers on plant B which bore only thrum flowers on other branches. Except in one case the flowers were distinctly either pin or thrum. Here again it seems very likely, though not certain, that the genotype of the embryo treated with thermal neutrons was Ss. If this is so, then the sector bearing pin flowers may have arisen through a mutation or conversion of S to s, through deletion of S, or through somatic crossing-over between the S locus and the kinetochore. Lefevre (12) and Whittinghill (20) found that gamma rays produce crossing-over in *Drosophila*. Similarly, results of James and Lee-Whiting (11) and of Roman and Jacob (17) indicate that ultraviolet radiation increases the frequency of somatic crossing-over in yeast. In *Aspergillus nidulans*, Fratello *et al.* (9) found no indication of increase in subchromosomal segregants after ultraviolet radiation, and Käfer (13), after 50,000 r of gamma radiation, recovered a high frequency of mitotic segregants, most of which arose by processes other than somatic crossing-over. Even if somatic crossing-over is higher after radiation, however, the method of origin of the sector bearing pin flowers remains uncertain.

The unusually high self-fertility of plant T of the progeny of plant B is quite interesting in view of the observed maintenance of both pollen and stylar reactions of its thrum flowers in crosses. If self-incompatibility is controlled by exactly the same gene or genes that control cross-incompatibility then these results cannot be valid. Accepting the results one can still postulate that part of the S locus may operate during selfing but not during crossing, which again leads us to a subdivision of a postulated S supergene. On the other hand, Beale (2) and Mather (15) have shown that the breakdown of heterostyly in *Primula sinensis* is due to two independent loci having pleiotropic effects rather than to changes in the S supergene. The recessive gene *a*, giving "Primrose Queen" eye, also shortens the style, affects the size of the stigmatic papillae and the incompatibility reaction of the style; the recessive gene *m*, giving "fertile double" flowers, raises the anther height, has no consistent effect on the style, and does not change the incompatibility reaction of the pollen. Using Darwin's method of comparative seed-setting, Mather and de Winton (14) concluded that apart from the major genes there must be a polygenic complex balancing the whole incompatibility system in

P. sinensis. They attributed the observed decline in incompatibility within the last century to selection by horticulturists of self-compatible pin plants —a view which was confirmed by Mather (15). Mather also pointed out that the "incompatibility reaction between pin and thrum has been weakened, but not eradicated, by horticultural selection in *P. sinensis*." Further support for such a hypothesis was provided by Rowlands' (16) work on field beans (*Vicia faba* L.). He concluded: "From the data presented, a system of self-incompatibility has been postulated which is largely based on polygenes, and which has developed by the breakdown of a once-efficient system through the effect of unconscious selection for uniformity throughout the past history of the crop."

It seems likely that there is an S supergene in buckwheat which was partially broken down in plant A, but we have no basis on which to decide whether its organization is exactly as postulated in the Introduction or not. It also seems quite possible that other genes acting on the self-compatibility locus have been affected by the radiation treatment in some of these buckwheat plants.

Acknowledgments

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References

1. ALTHAUSEN, L. 1908. Zur Frage über die Vererbung der langgriffeligen und kurzgriffeligen Blütenform beim Buchweizen und zur Methodik der Veredelung dieser Pflanze. Russ. J. exptl. Landwirtsch. **9**, 568.
2. BEALE, G. H. 1939. Further studies of pollen tube competition in *Primula sinensis*. Ann. Eugenics, **9**, 259–268.
3. DAHLGREN, K. V. O. 1922. Vererbung der Heterostylie bei *Fagopyrum* (nebst einigen Notizen über Pulmonaria). Hereditas, **3**, 91–99.
4. DARWIN, C. R. 1859. The origin of species. Dent & Sons Ltd., London. Chap IX. pp. 279–282.
5. DOWRICK, V. P. J. 1956. Heterostyly and homostyly in *Primula obconica*. Heredity, **10**, 219–236.
6. EGHIS, S. A. 1925. Experiments on the drawing up of a method of buckwheat breeding. Bull. Appl. Botany Genet. Plant Breeding, Leningrad, **14**(1), 235–251.
7. ERNST, A. 1957*a*. Austausch und Mutation im Komplex-gen für Blütenplastik und Inkompatibilität bei *Primula*. Z. Induktive Abstammungs- u. Vererbungslehre, **88**, 517–599.
8. ERNST, A. 1957*b*. Aberranten in Erbgängen der Heterostylie—Merkmale bei Primeln und ihre Bedeutung für Vererbungs- und Evolutionsprobleme. Arch. Julius Klaus-Stift. Vererbungsforsch. Sozialanthropol. u. Rassenhyg. **32**, 15–217.
9. FRATELLO, B., MORPURGO, G., and SERMONTI, G. 1960. Induced somatic segregation in *Aspergillus nidulans*. Genetics, **45**, 785–800.
10. GARBER, R. J. and QUISENBERRY, K. S. 1927. The inheritance of length of style in buckwheat. J. Agr. Research, **34**(2), 181–183.
11. JAMES, A. P. and LEE-WHITING, B. 1955. Radiation-induced genetic segregations in vegetative cells of diploid yeast. Genetics, **40**, 826–831.
12. LEFEVRE, G., JR. 1948. The relative effectiveness of fast neutrons and gamma rays in producing somatic crossing over in Drosophila. Genetics, **33**, 113.
13. KÄFER, E. 1960. High frequency of spontaneous and induced somatic segregation in *Aspergillus nidulans*. Nature, **186**, 619–620.
14. MATHER, K. and DE WINTON, D. 1941. Adaptation and counter-adaptation of the breeding system in *Primula*. Ann. Botany, N.S., **5**, 297–311.
15. MATHER, K. 1950. The genetical architecture of heterostyly in *Primula sinensis*. Evolution, **4**, 340–352.

16. ROWLANDS, D. G. 1959. Self-fertility in the field bean (*Vicia faba* L.). Heredity, **13**(1), 142.
17. ROMAN, H. and JACOB, F. 1958. A comparison of spontaneous and ultraviolet induced allelic recombination with reference to the recombination of outside markers. Cold Spring Harbor Symposia Quant. Biol. **23**, 155–159.
18. SAKNAROV, V. V. 1946*a*. Inheritance of the characters of heterostyly and the system of breeding in autotetraploid buckwheat. Compt. rend. acad. sci. U. S. S. R. **52**, 259–262.
19. TATEBE, T. 1953. Physiological studies on fertility in buckwheat. On self-fertile long styled individuals. Ikushugaku Zasshi Japan. J. Breeding, **2**, 240–244.
20. WHITTINGHILL, M. 1951. Some effects of gamma rays on recombination and on crossing over in *Drosophila melanogaster*. Genetics, **36**, 332–355.

THE EFFECT OF PHOTOPERIOD AND NITROGEN ON THE GROWTH AND DEVELOPMENT OF SEEDLINGS OF JACK PINE[1]

M. M. GIERTYCH[2] AND J. L. FARRAR[3]

Abstract

Nine seed lots of *Pinus banksiana* Lamb. and one of *P. contorta* var. *latifolia* Engelm. were grown for 115 days on five nitrogen levels and two photoperiods. The nitrogen levels were: zero N—no nitrogen, ¼N—51 p.p.m., ½N—102 p.p.m., 1N—203 p.p.m., and 2N—406 p.p.m. The two photoperiods were a long-night photoperiod (10-hour day) and a broken-night photoperiod (9.5-hour day followed by 0.5-hour light break in the middle of the dark period). The plants, five per vessel, were analyzed for their response to treatments in terms of growth, development, and nitrogen content.

(1) The breaking of the night doubled the height, total dry weight, and leaf weight and increased root weight and nitrogen absorption, but it lowered the number of fresh, active root tips, the proportion of the plant in root tissue, and the nitrogen concentration, inhibited production of secondary leaves, and delayed winter bud formation.

(2) The increase in nitrogen level in the medium lowered the percentage of fresh, active root tips, the proportion of the plant in root tissue, and the production of winter buds; it increased the content and concentration of nitrogen in the plants. Up to a certain maximum (about ½N), it increased the dry weight and height of the plants.

(3) The interrelation of growth and developmental stimuli is discussed. It is suggested that growth is an important factor in controlling development.

Introduction

It has long been recognized that the practice of silviculture creates need for information on the physiological characters of tree species (Kramer 1956). Jack pine is a species of considerable interest because of its wide distribution and comparatively rapid growth on a great variety of sites (Rudolf 1958). The responses of seedlings of jack pine to certain environmental factors are presented in this communication. One factor was the concentration of nitrogen in the nutrient medium. Nitrogen is an element which is frequently in short supply under natural forest conditions (Kramer and Kozlowski 1960). The second variable was photoperiod. The work of Wareing (1956) and Vaartaja (1959) among others has shown the striking effects of photoperiod on the physiological processes which control growth of tree seedlings.

Materials and Methods

The experimental material consisted of nine seed lots of eastern jack pine (*Pinus banksiana* Lamb.) and one of western jack pine (*P. contorta* var. *latifolia* Engelm.). The differential responses of these seed lots will be dealt with elsewhere; averages over all seed lots are presented here.

The seeds were germinated and grown in steel-plated vessels 10 cm tall and

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Contribution from the Department of Botany and the Faculty of Forestry, University of Toronto, Toronto, Ontario.
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6 cm in diameter using 50 seeds per vessel. The matrix was perlite—a porous form of silica. Drainage was provided by perforations in the vessel bottom. Continuous watering was achieved through a strip of filter paper extending from the perlite over the edge of the vessel into an adjoining vessel of water.

The nutrient solutions were adapted from those of Mitchell (1934, 1939). The principal mineral elements (P, 151 p.p.m.; K, 320 p.p.m.; Ca, 244 p.p.m.; Cl, 436 p.p.m.; S, 280 p.p.m.; Mg, 173 p.p.m.; and Fe, 3 p.p.m.) were supplied as KH_2PO_4, K_2SO_4, $CaCl_2$, $MgSO_4$, and iron chelates. Nitrogen was provided as NH_4NO_3 and varied to give five levels as follows:

Code	Zero N	¼N	½N	1N	2N
p.p.m.	0	51	102	203	406

A preliminary experiment had suggested that 203 p.p.m. was in the region of optimum level. The nutrient solution was given at the rate of 50 ml per week applied at 3- or 4-day intervals directly into the growth vessel.

Two photoperiods were arranged. In one photoperiod the light period was 10 hours and the dark period 14 hours; this will be referred to as the 'long-night photoperiod'. The other had a similar light period of 9.5 hours, but the night was broken into two 7-hour periods by a 0.5-hour light break; this will be referred to as the 'broken-night photoperiod'.

The two photoperiods each provided the same amount of light—an intensity of about 700-foot candles at plant level for a period of 10 hours a day. The light was provided by tungsten filament lamps and filtered by 2 cm of flowing water and a sheet of plate glass.

The experiment was conducted in two growth chambers corresponding to the two photoperiods. The growth chambers provided a constant circulation of air at a temperature of 20° C and a relative humidity of about 75%. The air was changed about every 3 seconds, and about 20% filtered outside air was added at each change. There were two replicates in each growth chamber.

As the experiment progressed, the number of plants was gradually reduced from 50 to 5 per vessel by removing sick plants and others randomly selected. The plants were harvested 115 days after sowing, which was an average of about 107 days after germination. Each plant was measured for height from medium surface to stem apex and scored for presence of secondary leaves (needle bundles), dormant buds, and fresh, active root tips. For each group of plants from one vessel, dry weight was determined for foliage, roots, and stem; these values were reduced to weight per plant.

The nitrogen determinations were made by the Kjeldahl method (Piper 1950) using metallic selenium with K_2SO_4 (1:30) as catalyst. Results were expressed as a proportion of dry weight and as milligrams per plant.

Data were analyzed by the variance procedure (Snedecor 1956). All effects discussed were significant at the 5% level.

Results

Height

The effect of treatment on height is shown in Fig. 2. There was a very strong response to photoperiod except where no nitrogen was added to the solution. Increase in height due to the breaking of the night was primarily due

to an increase in the length of the stem above the cotyledonary node, the hypocotyl remaining of about the same height as for the plants grown under the long-night photoperiod (Fig. 12). The increase in height due to the addition of nitrogen was most evident under the broken-night photoperiod. There was little difference in height at nitrogen levels from ½N to 2N (Fig. 11).

Total Dry Weight

The effect of the treatments on the dry weight is shown in Fig. 1. As with height, the breaking of the night almost doubled dry weight when nitrogen was present. The effects of nitrogen were evident under both photoperiods. The two higher levels of nitrogen were not superior to the ½N.

Roots

The dry weight of roots in milligrams and as a proportion of total dry weight is shown in Fig. 3 and 4. The broken-night photoperiod increased the weight of roots, but decreased the proportion of the assimilate which was used in root growth. Above the zero N, nitrogen level had very little effect on root weight, but reduced the proportion of roots. The increasing level of nitrogen decreased the number of plants with fresh, active root tips. The breaking of the night also decreased the number of plants with fresh, active root tips (Table I). The root tips not fresh and active were suberized or swollen.

Foliage

The dry weights of leaves in milligrams and as a proportion of total dry weight are shown in Figs. 5 and 6. The broken-night photoperiod increased both the amount and proportion of foliage. Up to ½N, nitrogen level increased both amount and proportion of foliage. The higher levels differed little from ½N.

Secondary leaves (needle bundles) were absent under the broken-night photoperiod and in the zero-N level (Table I).

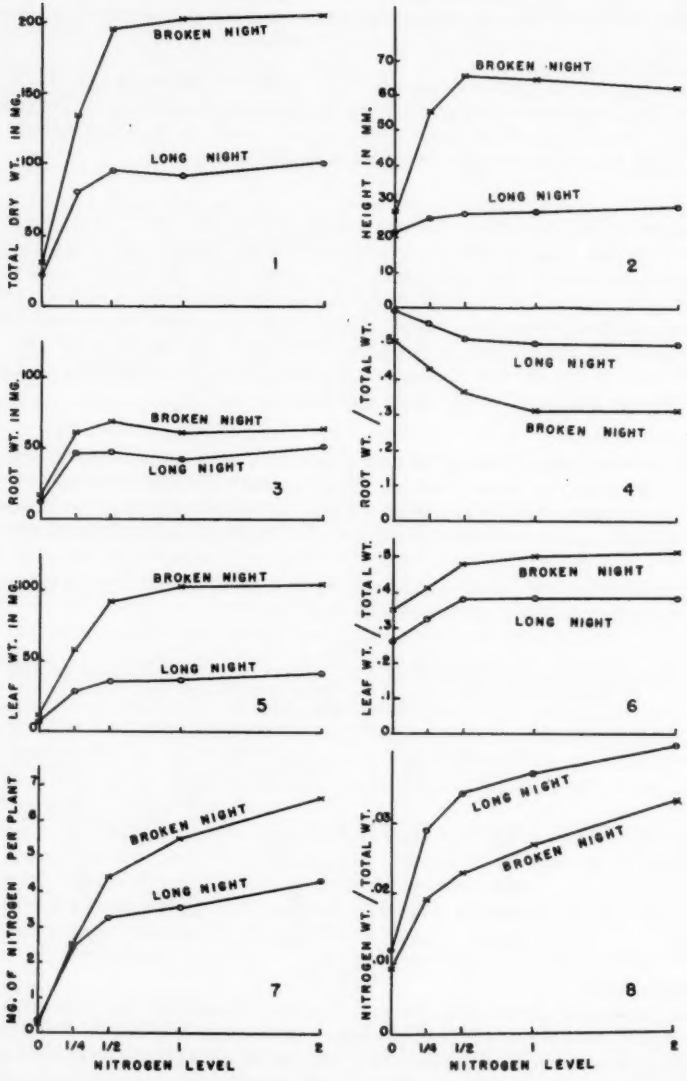
TABLE I

Percentage of plants with fresh root tips, secondary leaves, and winter buds in relation to level of nitrogen and photoperiod (long night, L, and broken night, B)

	% of plants with fresh root tips		% of plants with secondary leaves		% of plants with winter buds	
N level	L	B	L	B	L	B
Zero N	79	100	0	0	3	2
¼N	99	98	44	0	52	6
½N	81	59	41	0	30	3
1N	77	15	42	0	31	3
2N	64	13	56	0	28	1
Average	80.0	57.0	36.5	0	28.8	3.0

Dormant Buds

The number of plants with dormant buds at the end of the experiment was considerably less under the broken-night photoperiod and at zero N (Table I). For the remaining N levels there was a decrease in number of plants with winter buds with increase in N level. In Fig. 10 is shown the inverse relation that


TOTAL DRY WT. IN MG.
BROKEN NIGHT
LONG NIGHT
1
HEIGHT IN MM.
BROKEN NIGHT
LONG NIGHT
2
ROOT WT. IN MG.
BROKEN NIGHT
LONG NIGHT
3
ROOT WT. / TOTAL WT.
LONG NIGHT
BROKEN NIGHT
4
LEAF WT. IN MG.
BROKEN NIGHT
LONG NIGHT
5
LEAF WT. / TOTAL WT.
BROKEN NIGHT
LONG NIGHT
6
NO. OF NITROGEN PER PLANT
BROKEN NIGHT
LONG NIGHT
7
NITROGEN WT. / TOTAL WT.
LONG NIGHT
BROKEN NIGHT
8
0 1/4 1/2 1 2
NITROGEN LEVEL
0 1/4 1/2 1 2
NITROGEN LEVEL

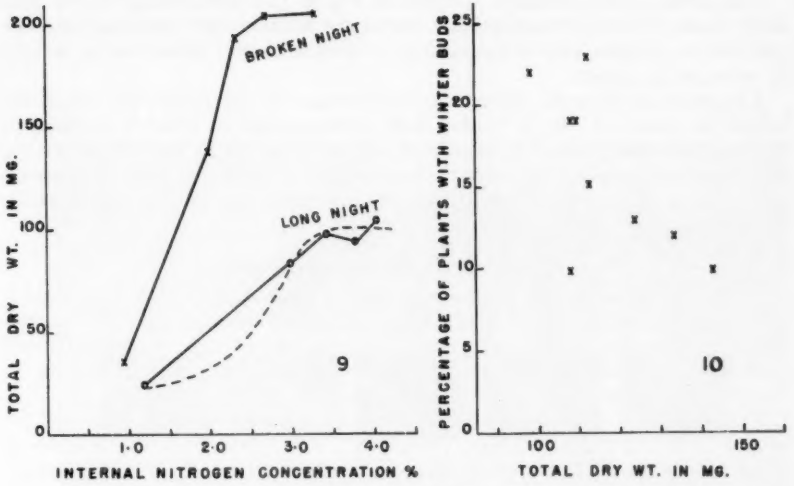


FIG. 9. The relation between dry weight and internal nitrogen concentration for the two photoperiods.

FIG. 10. The relation of dry weights of plants to the percentage of plants with winter buds.

existed between dry weight and presence of dormant buds for the different seed lots.

Nitrogen Content

The weight of nitrogen per plant is shown in Fig. 7. At the higher levels of nitrogen, there was much more nitrogen per plant under the broken-night photoperiod. The response to nitrogen was very strong at the lower levels and continued at a reduced rate up to the highest level.

It may be noted here that the average weight of nitrogen per seed was 0.17 mg, and the average weight of nitrogen in the plants grown at zero N was 0.30 mg. The additional 0.13 mg came from the nongerminating seed and seed coats, and other contaminants; the amounts were small compared with the totals.

FIG. 1. The influence of photoperiod and nitrogen level on total dry weight.

FIG. 2. The influence of photoperiod and nitrogen level on height.

FIG. 3. The influence of photoperiod and nitrogen level on root weight.

FIG. 4. The influence of photoperiod and nitrogen level on the ratio of root weight to total weight.

FIG. 5. The influence of photoperiod and nitrogen level on leaf weight.

FIG. 6. The influence of photoperiod and nitrogen level on the ratio of leaf weight to total weight.

FIG. 7. The influence of photoperiod and nitrogen level on the nitrogen content per plant.

FIG. 8. The influence of photoperiod and nitrogen level on the ratio of weight of nitrogen to total weight.

The nitrogen concentration is shown in Fig. 8. The outstanding feature here is the lower nitrogen concentration under the broken-night photoperiod. The relation to nitrogen level of the medium is similar to that shown by the weight of nitrogen per plant.

The relation between nitrogen concentration in the plant and total dry weight is shown in Fig. 9. Under both photoperiods an internal increase in nitrogen concentration was associated with an increase in dry weight at the lower concentrations. The curves appear to flatten off at the higher concentrations. For a given concentration there was a much greater dry weight under the broken-night photoperiod.

Discussion and Conclusions

The data indicated that photoperiod was of primary importance in regulating the life of jack pine seedlings. Breaking the 14-hour dark period into two 7-hour periods doubled the dry weight of the plants and their height, increased nitrogen absorption, prevented the formation of adult foliage (needles in fascicles), and reduced the formation of dormant buds.

The responses of these plants were mainly similar to those observed by others (Wareing 1956; Vaartaja 1954, 1957, 1959). However, the absence of adult foliage on the larger plants exposed to the light break, and its presence on the smaller plants grown under long nights, seems to be a new observation. The association of adult foliage with dormant buds has been observed previously by one of us (J.L.F.), but the presence of both on such small plants was unexpected.

The appearance of needles in fascicles is a measure of progress of physiological maturation, whereas the appearance of dormant buds is a measure of the progress of seasonal development. Breaking of the night hindered both processes. The mechanism by which the effect of the breaking of the night operates is a topic of considerable speculation (Hendricks and Borthwick 1959; Black and Wareing 1960). The photoperiodic treatments were such that the same amount of light energy was provided for all the plants. Only the length of the night was cut by splitting it in two. Thus the differences were caused by a mechanism not dependent on energy supply. Such mechanisms are known to exist (Hendricks and Borthwick 1959). Borthwick and Hendricks (1960) have demonstrated in some species the presence of a photosensitive pigment in two interconvertible phases, and believe that the condition of the pigment affects the metabolism. A similar system may be operative in jack pine.

The net effect on growth is of two kinds. One is the prolongation of the actual period of growth, as is evidenced by the delay of dormant bud formation. This, however, does not explain all. From the results, it is obvious that when the plants were growing, their growth rate was much higher under the broken-night photoperiod. (This was confirmed by measurements made on the thinnings collected periodically during the course of the experiment.)

As the growth increase was primarily in the stem and foliage, it is likely that the mechanism is hormonal in nature, auxins being known to promote stem elongation more readily than root growth (Van Overbeek 1959).

While promoting growth phenomena, the broken-night photoperiod seems to have an opposite effect on developmental phenomena (Ashby 1950). The delay

PLATE I

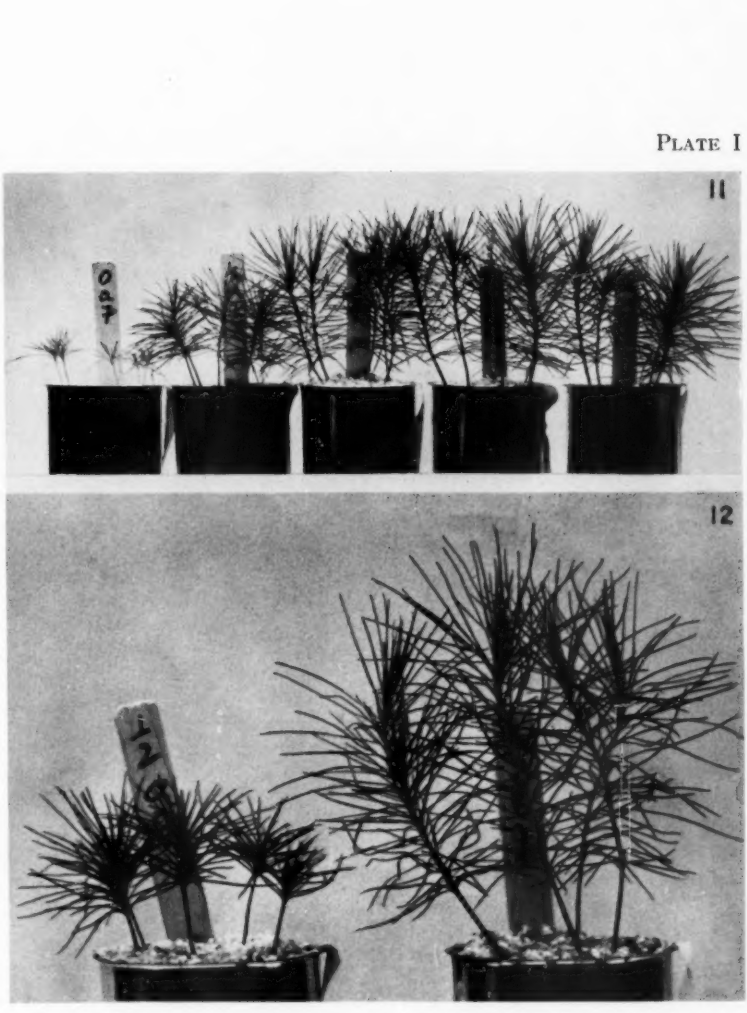


FIG. 11. The appearance of plants grown under broken-night photoperiod and varying nitrogen level (increasing from left to right).

FIG. 12. The effect of photoperiod on plants grown on a nitrogen level of $\frac{1}{2}$N. The plants on the right were grown under the broken-night photoperiod.

in winter bud formation and inhibition of secondary leaf production suggests that the broken-night photoperiod was not favorable to development. It is possible that the mechanism operates through its effect on growth. Development depends on growth—it will not take place without sufficient growth, as the plants grown on zero N demonstrate, whereas growth without development is possible for an indefinite period as in tissue cultures. But it is also likely that development will not proceed when growth is excessive, that is optimum conditions for growth may retard physiological aging. Unless meristematic cells are sufficiently mature physiologically, they will not produce tissues we would recognize as characteristic of a later developmental stage. Krenke (1940) believed that rapid growth continuously rejuvenates meristematic cells, thus preventing their physiological maturation.

The increase in the root ratio under the long-night photoperiod is in agreement with a number of observations concerning the effect of photoperiod on relative size of tops and root systems (Sinnott 1960). However, Wareing (1950) reported that photoperiod had little effect on the root system of Scots pine. In our experiment the light break resulted in a larger root system, but the roots formed a lower proportion of the entire plant (Figs. 3 and 4). The light break also reduced the number of plants with fresh, active root tips (Table I).

Under lower nitrogen levels, the proportion of roots was higher, and more plants had fresh, active root tips. Thus under conditions of nitrogen shortage roots played a more important part in the plant metabolism.

The combined effects of photoperiod and nitrogen nutrition are of interest. While the amount of nitrogen absorbed per plant was increased by the light break, the proportion of nitrogen in the plant was reduced (Figs. 7 and 8). It might be said that the plants exposed to the light break used the absorbed nitrogen more efficiently. Similarly, an increase in nitrogen concentration in the plants under the broken night was associated with a much greater proportionate increase in dry weight than under the long night (Fig. 9).

The increase in dry weight resulting from an increase in the nitrogen level of the external medium followed the law of diminishing returns—each increment of nitrogen produced less effect than the previous one (Fig. 1). The effects of increasing nitrogen were more pronounced under the more favorable photoperiod—an observation which fits the concept of limiting factors (as summarized by Meyer and Anderson 1952, pp. 335, 612).

Macy (1936), supported by Ulrich (1952), suggested the concept of critical internal concentrations of certain elements below which yield is related to concentration (the region of poverty adjustment), and above which yield is not affected by concentration (the region of luxury consumption). The data from the plants under the broken-night photoperiod fitted that concept with regard to nitrogen (Fig. 9). Up to about 2.5% nitrogen, an increase in concentration was associated with an increase in dry weight; above 2.5% the curve flattens. The data from the plants under the long-night photoperiod could also be interpreted to indicate a critical concentration below 3%. In his work with *Pinus sylvestris* and *P. strobus* Mitchell (1934 and 1939) found that the optimum internal nitrogen content of the plants ranged from 2.50 to 3.25%. Swan's (1960) data show increasing size of plant up to a concentration of 2.6% nitrogen—the highest attained.

Information on the effect of nitrogen on the developmental processes is very scanty. The decrease in winter bud formation associated with a nitrogen level (Table I) probably operated through the effect of the nitrogen level on growth. Otherwise the only effect of nitrogen level is that complete lack of nitrogen results in starvation of the plants with an absence of bud formation and production of secondary leaves.

While the container size and the frequency of adding the nutrient solution might seem inadequate compared with those used by other investigators, the size and appearance of the seedlings indicated that they had developed without serious hindrance. The chief limiting factor was the low intensity of the light source.

Acknowledgments

We are grateful to Mr. Mark Holst, Petawawa Forest Experiment Station, Department of Forestry, Chalk River, Ont., for providing the seeds used in this experiment; the Ontario Research Foundation for financing a major part of the project; and the Department of Botany, University of Toronto, for making available the growth chambers and other facilities in the departmental building.

References

ASHBY, E. 1950. Studies in the morphogenesis of leaves. VI. Some effects of length of day upon shape in *Ipomoea caerulea*. New Phytologist, **49**, 375–387.

BLACK, M. and WAREING, P. F. 1960. Photoperiodism in the light inhibited seed of *Nemophila insignis*. J. Exptl. Botany, **11**, 28–39.

BORTHWICK, H. A. and HENDRICKS, S. B. 1960. Photoperiodism in plants. Science, **132**, 1223–1228.

HENDRICKS, S. B. and BORTHWICK, H. A. 1959. Photocontrol of plant development by the simultaneous excitation of two interconvertible pigments. Proc. Natl. Acad. Sci., N.S., **45**, 344–349.

KRAMER, P. J. 1956. The role of physiology in forestry. Forestry Chronicle, **32**, 297–307.

KRAMER, P. J. and KOZLOWSKI, T. T. 1960. Physiology of trees. McGraw-Hill Book Co., New York.

KRENKE, N. P. 1940. The theory of cyclic senescence and rejuvenation in plants and its practical application. Gossudarstwiennoje Izdatielstwo Koloznej Sovhoznoj Literatury, Moscow, Selhozgiz. *Translated by* Imp. Bur. Plant Breeding and Genet., School of Agr., Cambridge.

MACY, P. 1936. The quantitative mineral nutrient requirements of plants. Plant Physiol. **11**, 749–764.

MEYER, B. S. and ANDERSON, D. B. 1952. Plant physiology. 2nd ed. Van Nostrand Co., Princeton, New Jersey.

MITCHELL, H. L. 1934. Pot culture tests of forest soil fertility. Black Rock Forestry Bull. 5.

——— 1939. The growth and nutrition of white pine (*Pinus strobus* L.) seedlings in cultures with varying nitrogen, phosphorus, potassium, and calcium. Black Rock Forestry Bull. 9.

PIPER, C. S. 1950. Soil and plant analysis. Interscience Publ. Inc., New York.

RUDOLF, P. O. 1958. Silvical characteristics of jack pine. Sta. Papers Lake States For. Exptl. Sta. 61.

SINNOTT, E. W. 1960. Plant morphogenesis. McGraw-Hill Book Co., New York.

SNEDECOR, G. W. 1956. Statistical methods. 5th ed. Iowa State College Press, Ames, Iowa.

SWAN, H. S. D. 1960. The mineral nutrition of Canadian pulpwood species. Can. Pulp and Paper Research Inst. Tech. Rept. No. 168.

ULRICH, A. 1952. Physiological bases for assessing the nutritional requirements of plants. Ann. Rev. Plant Physiol. **3**, 207–228.

VAARTAJA, O. 1954. Photoperiodic ecotypes of trees. Can. J. Botany, **32**, 392–399.

——— 1957. Photoperiodic responses in seedlings of northern tree species. Can. J. Botany, **35**, 133–138.

——— 1959. Evidence of photoperiodic ecotypes in trees. Ecol. Monographs, **29**, 91–111.

VAN OVERBEEK, J. 1959. Auxins. Botan. Rev. **25**, 269–350.

WAREING, P. F. 1950. Growth studies in woody species. I. Photoperiodism in first year seedlings of *Pinus sylvestris*. Physiol. Plantarum, **3**, 258–276.

——— 1956. Photoperiodism in woody plants. Ann. Rev. Plant Physiol. **7**, 191–214.

THE LIGHT COMPENSATION POINTS OF SOME HERBACEOUS PLANTS INSIDE AND OUTSIDE DECIDUOUS WOODS IN GERMANY[1]

H. LIETH[2] AND D. H. ASHTON[3]

Abstract

The light compensation points and respiration rates of cut leaves of *Ranunculus ficaria*, *Anemone nemorosa*, and *Allium ursinum* were determined at 18–19° C at intervals throughout the growing season of 1959 from plants grown inside and outside the forest. The light compensation points of leaves from inside and outside the forest decreased progressively during the growing season from March to June, but showed two sharp increases following severe frosts in the mid-season. The compensation points of plants in the forest were consistently lower than those in the open, and showed a smaller increase due to frost damage. The drift in light compensation points could in most cases be correlated with a decrease in the dark-period respiration rate. However, the marked exceptions which occurred after the frosts were presumably due to a decrease in the efficiency of photosynthesis.

The light compensation points of *Allium* were consistently lower than those of *Anemone*, which were in turn frequently lower than those of *Ranunculus*. Field observations suggested that *Allium* was the most tolerant of shade conditions and persisted above ground for the longest period under the forest canopy. When the light compensation points of these species were compared on the basis of equal respiration rates, *Allium* was lowest and presumably had the most efficient photosynthetic apparatus and *Anemone* was highest and presumably had the least efficient.

Introduction

Recently Lieth and Vogt (1959) showed that there was a seasonal march of the light compensation points in several forbs in the deciduous woods of southwestern Germany. The light compensation points of these plants were high in early spring and decreased steadily to a minimum value in the late spring and early summer. This appeared to be correlated with the bud burst of the tree canopy and the gradual development of full shade, although at that time no measurements of light intensity were made.

It has also been shown by Boysen-Jensen (1932), Lieth and Vogt (1959), and Lieth (1960) that, as expected, there is a general correlation between the light compensation point and the dark-period respiration rate. However, exceptions may sometimes occur as a result of severe frosts when the light compensation point increases greatly without any corresponding increase in the dark-period respiration rate (Pavletić and Lieth 1958).

The present study was undertaken to verify the seasonal march of the light compensation points inside and outside the forest, and to correlate these with the dark-period respiration rate and the daily light intensity.

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Contribution from Botanisches Institut der Landwirtschaftlichen Hochschule, Stuttgart-Hohenheim. This paper has been supported by the DAAD (German Academic Exchange Service), Bonn.
[2]Botanisches Institut der Landwirtschaftlichen Hochschule, Stuttgart-Hohenheim. National Research Fellow 1960/61 at the Institute Botanique, Université de Montréal.
[3]Botany Department, University of Melbourne, Parkville N. 2, Australia.

Materials and Methods

The light compensation point was measured colorimetrically according to the method described by Lieth (1958, 1960). The method is based on the sensitivity of cresol red indicator to small changes in the partial pressure of CO_2 in the atmosphere (Kauko and Carlberg 1935). The indicator solution used consists of 10 mg/l. cresol red,[4] 84 mg/l. $NaHCO_3$, and 7.46 g/l. KCl. Cut leaves were mounted in rubber-stoppered test tubes (20×3 cm) so that the upper surface received maximum illumination from a high-pressure mercury lamp. Eight to ten test tubes were placed at different distances from the lamp on a gently sloping ladder. The position or region along the ladder which showed no color change in the indicator solution from that of the controls after a period of 2–3 hours was taken to be the light compensation point and the illumination was measured in lux.

The respiration rate of cut leaves in the dark was measured at the same time, and in the same room as the experiments on the light compensation point. An infrared gas analyzer (URAS Badische Analin and Sodafabrik) with an open, through-flow switching system was used and the results expressed as changes in the volume of CO_2 per mg dry weight of leaf.

Three species of forbs were investigated: *Allium ursinum* L., *Anemone nemorosa* L., and *Ranunculus ficaria* L. The area chosen for study was in a broad forest border of *Fagus sylvatica* L., *Carpinus betulus* L., and *Fraxinus excelsior* L., and an adjacent open meadow area in the Botanical Garden of the Agricultural College at Stuttgart-Hohenheim.

In very early spring, before the leaves of the forbs had developed, stocks of each species were carefully transplanted with intact soil from the forest floor to a prepared bed in the meadow site about 50 meters from the forest border. Experiments were commenced about 3 weeks later when the first leaves began to develop. Leaf samples were cut from several plants at both sites, placed between moist filter paper, and immediately taken to the laboratory. The procedures were standardized in order to minimize variability. Since the light compensation point varied somewhat during the course of the day, comparative samples were taken at 9 a.m. Only fully expanded leaves formed early in the growing season were sampled, and the same regions of the leaf were tested each time. For *Allium* only the better-illuminated upper one-third of the leaf was taken, for *Anemone* all of three main leaf lobes were used, and in *Ranunculus* the whole leaf lamina was used. Since the light compensation point is simply the light intensity at which photosynthesis balances respiration, it was not necessary to have specimens of exactly the same size in each tube.

During the whole course of the investigation the light intensity at the experimental plots both outside and inside the forest border was continuously recorded by cosine-corrected selenium cells attached to a recording meter (Firma Dr. B. Lange, Berlin). The daily percentage of light reaching the shaded locations was then calculated from the planimeter-measured area under the curve. Following the terminology of Wiesner (1907) this is called the *Lichtgenuss*. The seasonal variation of the Lichtgenuss during this period is shown with the light compensation points in Fig. 1.

[4]Cresol red (Kresolrot) is Merck's trade name for σ-cresolsulphophthaleine.

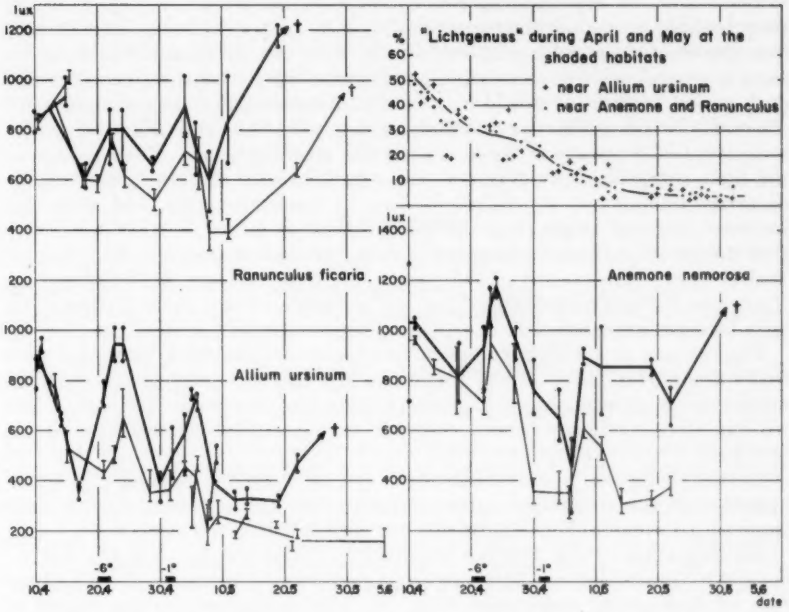

FIG. 1. Light compensation points during the spring of 1959 for three species. Abscissa shows date of measurement. Ordinate is in lux (1 lux = ca. 0.1 ft-c). Each thick line follows the mean of the measured compensation points under unshaded conditions. Each thin line follows this mean under shaded conditions. † indicates time of death. Frost periods are indicated along the abscissa with the minimum temperature from a nearby weather station.

The upper right part of the figure shows the percentage of daylight received (Lichtgenuss) by the shaded plants during the time of the investigation.

Results

The light compensation points of the leaves of all three species decreased from early spring to the onset of senescence in late spring or early summer, when there was a spectacular increase. This behavior was similar to that found by Lieth and Vogt (1959) under forest canopy, but in the present experiments it also occurred in the plants growing in the open. It would appear therefore that the decrease in the compensation point of these spring-flowering geophytes is due to some internal rhythm affecting the balance of photosynthesis and respiration rather than a progressive change in the environmental factors. As was expected from the work of Boysen-Jensen (1932) and Ashton (1956) the light compensation points of plants grown in the open were consistently higher than those of plants grown under shaded conditions, and this was especially true when the Lichtgenuss fell below 30%. The curves also show two remarkable peaks which follow the incidence of two severe frost periods on April 20–21 and May 1–2, 1959.

The results of the respiration measurements are shown in Fig. 2, where they are plotted against the corresponding light compensation points. In each case the results for inside and outside the forest are combined. These results show a general positive correlation between the light compensation point and dark-period respiration for *Allium* and *Ranunculus*, but no correlation for *Anemone*. It is possible that this discrepancy could have been due to a greater sensitivity of *Anemone* to frosts during the growing season. The increase in the light compensation points immediately following the two frost periods occurred without any significant increase in respiration rate, and since this increase persisted longer than the actual period of frosty weather it is likely that the photosynthetic metabolism of these species was temporarily damaged. Walter (1929) and Levitt (1956) point out that in some sensitive plants, frosts and the accompanying wilting can seriously reduce photosynthesis and even increase respiration.

The increase in the light compensation point was greater in the open than under the partial shade of the forest canopy, and was least of all under an understory of *Sambucus nigra* L., where there was no damage and only slight wilting. More studies are needed, however, to fully explain the deviation shown in Fig. 2.

In comparing the relative behavior of the three species examined, it would appear that the respiration rates of *Ranunculus* are in general higher than those of *Anemone* and *Allium*, which are approximately the same.

An indication of the relative tolerance of these species to shade can be gauged by examining the light intensity necessary to compensate for a given rate of dark-period respiration (Fig. 2). This is highest for the leaves of *Anemone*, intermediate for those of *Ranunculus*, and least for those of *Allium*. This is to some extent corroborated by field observations, where *Allium*

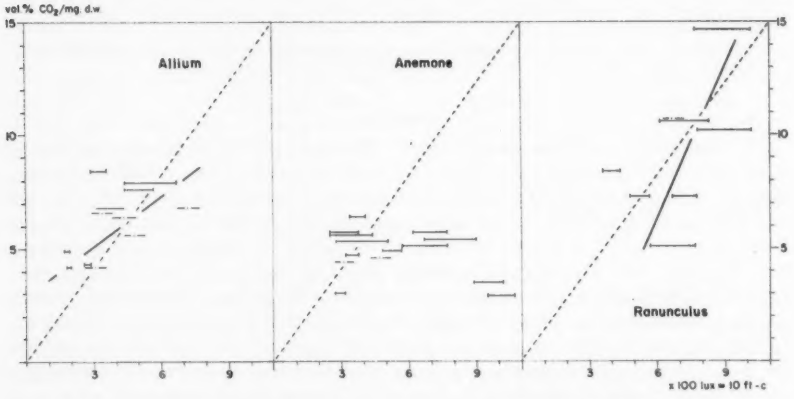

FIG. 2. Light compensation range plotted against dark-period respiration. Abscissa in lux, ordinate in volume per cent CO_2 per mg dry weight. The dotted diagonal line represents a constant ratio. The greater the efficiency of the assimilation apparatus, the more the compensation values appear to the left of the dotted line. For further explanation see text.

not only grows under deeper shade than the other species but is capable of persisting for a longer period under the fully expanded canopy of the forest.

The deductions which can be made, therefore, on the relative behavior of these species are approximately as follows:

Respiration rates: *Ranunclus* > *Anemone* = *Allium*,

Compensating light intensity at equal respiration rates:

Anemone > *Ranunculus* > *Allium*,

Light compensation points: *Ranunculus* > *Anemone* > *Allium*.

References

ASHTON, D. H. 1956. Studies on the autecology of *Eucalyptus regnans* F. v. M. Ph. D. Thesis, Melbourne.

BOYSEN-JENSEN, P. 1932. Die Stoffproduktion der Pflanze. Jena, Fischer Verlag.

KAUKO, Y. AND CARLBERG, J. J. 1935. Praktische Ausführung der Kohlensäurebestimmung in Gasgemischen mit Hilfe von pH-Messungen. Z. anal. Chem. **102**, 393–407.

LEVITT, J. 1956. The hardiness of plants. Academic Press, New York.

LIETH, H. 1958. Grenzen und Anwendungsmöglichkeiten der colorimetrischen CO_2-Bestimmung. Planta, **51**, 705–721.

——— 1960. Über den Lichtkompensationspunkt der Landpflanzen I and II. Planta, **54**, 530–554 and 555–576.

LIETH, H. AND VOGT, M. 1959. Der Lichtkompensationspunkt einiger Waldschattenpflanzen im Sommer und im Früjahr. Proc. IXth Intern. Botan. Congr., Montreal. Vol. II. pp. 227–228.

PAVLETIĆ, Z. AND LIETH, H. 1958. Der Lichtkompensationspunkt einiger immergrüner Pflanzen im Winter und im Frühjahr. Ber. deut. botan. Ges. **71**, 309–314.

WALTER, H. 1929. Die osmotischen Werte und die Kälteschäden unserer wintergrünen Pflanzen während der Winterperiode. Ber. deut. botan. Ges. **47**, 338–348.

WIESNER, J. 1907. Der Lichtgenuss der Pflanzen. Leipzig.

TRIGLOCHIN GASPENSE, A NEW SPECIES OF ARROW GRASS[1]

D. LÖVE[2] AND H. LIETH[3]

Abstract

A new species, *Triglochin gaspense* Lieth & D. Löve, is described from a salt marsh between Barachois-Ouest and Coin-du-Banc, Gaspé Peninsula, Quebec, Canada. It is distributed in Quebec, the Canadian Maritime Provinces, and Newfoundland, as well as in at least the northern part of the state of Maine, U.S.A. It is well differentiated from the other American species of the *Triglochin maritimum* complex, to which it belongs, morphologically (5–20 cm tall, leaves overtopping the short, few-flowered spike), cytologically ($2n = 96$ chromosomes), and ecologically (confined to the tidal zone of the Atlantic coast below the high-water mark). The type specimen of the new species is preserved in the Marie-Victorin Herbarium, Université de Montréal.

Introduction

In July 1902, M. L. Fernald, G. G. Kennedy, J. F. Collins, and E. F. Williams visited Schooner Bay at Cutler, Maine. There, in the tidal zone below the high-water mark, they found a dwarfish form of *Triglochin* with racemes only 2 to 6 cm long. Closer investigation revealed that the number of carpels in this form was three to six instead of the normal six. Later, Fernald and Williams came across a similar, small *Triglochin* at the mouth of the Tête-à-Gouche River, Gloucester Co., New Brunswick. The plants there had the normal number of carpels, even though they were fruiting when only 3 to 6 cm tall. Fernald (1903) ascribed their small size to the adverse ecological condition: "twice a day it was entirely covered by the chilling ocean water". But he could not be certain whether the habitat also affected the number of carpels, since normal, tall *T.* "*maritima*" (probably = *T. elatum* Nutt.) from warmer habitats of the Tête-à-Gouche marshes occasionally showed a similar reduction in carpel number (Fernald *loc. cit.*).

In his paper, Fernald did not give the dwarf type any particular taxonomical rank, as he considered it but a phenotypical aberrant. However, in the 8th edition of Gray's Manual (Fernald 1950), he apparently had this type in mind under *T. maritimum* L. as the following sentence indicates: "The smallest diffuse individuals with open raceme are *T. concinna* Davy, hardly a distinct species." Therefore, one occasionally comes across herbarium material of small *Triglochin* specimens from the coast of the Canadian Maritimes under the name of *T. concinnum* Davy.

In 1956 the small *Triglochin* type was observed and collected at the mouth of Bonaventure River, Gaspé Peninsula, Quebec, by A. and D. Löve. This material did, however, not survive transplantation. Later, fresh material as well as dried specimens were brought to them by Dr. E. Dahl of Norway, who

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Contribution from the Institut Botanique, Université de Montréal, Montreal, Que.
[2]Institut Botanique, Université de Montréal.
[3]Botanisches Institut der Landwirtschaftlichen Hochschule Stuttgart-Hohenheim, Germany. National Research Council Fellow, 1960/61, Université de Montréal.

collected in the Gaspé Peninsula in 1958, and found the plant in some new localities. This time we succeeded in keeping the plants alive in the greenhouse at the Botanical Institute. They did not fare well, and attempts to study their chromosomes in the root tips proved futile.

In 1960, H. Lieth, working with Drs. P. Dansereau and L. Levesque in Gaspé, found additional localities for this interesting dwarf taxon of *Triglochin*. Furthermore, Lieth studied its ecological habitat in detail. Upon his return to Montreal in September 1960, he was able to recondition the soil of our cultivated plants by correcting the salinity with the result that the plants improved to the extent that we were able to secure fresh and actively growing root tips. The chromosome number proved to be $2n = 96$ (Fig. 2), a number not previously reported for the genus *Triglochin* from the east coast of North America. This and other facts discussed below indicate that we have reason to consider these plants as belonging to a new species.

Morphology

In every respect, it is evident that the new taxon (Fig. 1) belongs to the *T. maritimum* complex. A step-by-step comparison between it and other North American members of this group follows.

Size

The Atlantic plants reach a height of only 5 to 20 cm in fully grown specimens, 10 to 15 cm being apparently their normal size (Fig. 1). This is in sharp contrast with the other species of the *T. maritimum* complex, which usually reach 50 to over 100 cm in height.

Root System

The rhizome is rather short and slender (Fig. 1). The plants form low, even, "lawn"-like patches in the marsh contrasting with the condensed, turflike clumps formed by tall *T. elatum*. The bases of the leaves and stems of our taxon are covered with fibrous remnants of old sheaths. These, however, do not persist for nearly as long a time as the corky-indurate and fibrous sheaths of *T. elatum*. The base of the small plants, therefore, appears considerably more slender than in *T. elatum* and is occasionally somewhat bulblike, in this respect reminiscent of both *T. striatum* R. & P. and *T. concinnum*.

Leaves

The leaves of the dwarf plant are very slender, almost terete, ½–1 mm in diameter, and curve outwards from the sheath at an angle of 45–50° (Fig. 1). This is easily visible in fresh material, but less so in pressed specimens, where sometimes only a slight compression of the longitudinal ridges of the leaf near the ligule may indicate the bent area. In all other American *Triglochin* the sheath runs almost directly into the erect blade.

The leaves grow as tall as, or taller than, the scapes of the small taxon and end in a point. In the other American species of the *T. maritimum* complex the scapes extend beyond the leaves, often considerably so.

The ligule in the dwarf plants is short, ½ to barely 1 mm long, and sometimes slightly hood-shaped (Fig. 2*a*). In *T. concinnum* (Fig. 2*c*), the ligule is likewise cucullate and often attached at the middle, giving the impression of being

Herbier Marie-Victorin Institut Botanique Université de Montréal
PLANTES DE LA GASPESIE, CANADA
Triglochin gaspense Lieth & D. Löve
No d'herbier: 60-0626 Date: July 25, 1960
No d'herborisation*: 600725-0555
Habitat: salt-marsh below high-water mark.
(Specimen at lower right with abnormal, reduplicated carpels; normal number of carpels is 6.)
Localité: Between Barachois-Ouest Comté: Gaspesie and Coin-du-Banc
Leg.: P. DANSEREAU, H. LIETH, B. GALTZ, R. GUILLETTE,
Det.: D. Löve and H. Lieth, May 1961
TYPUS

FIG. 1. Type specimen of *Triglochin gaspense* Lieth & D. Löve.

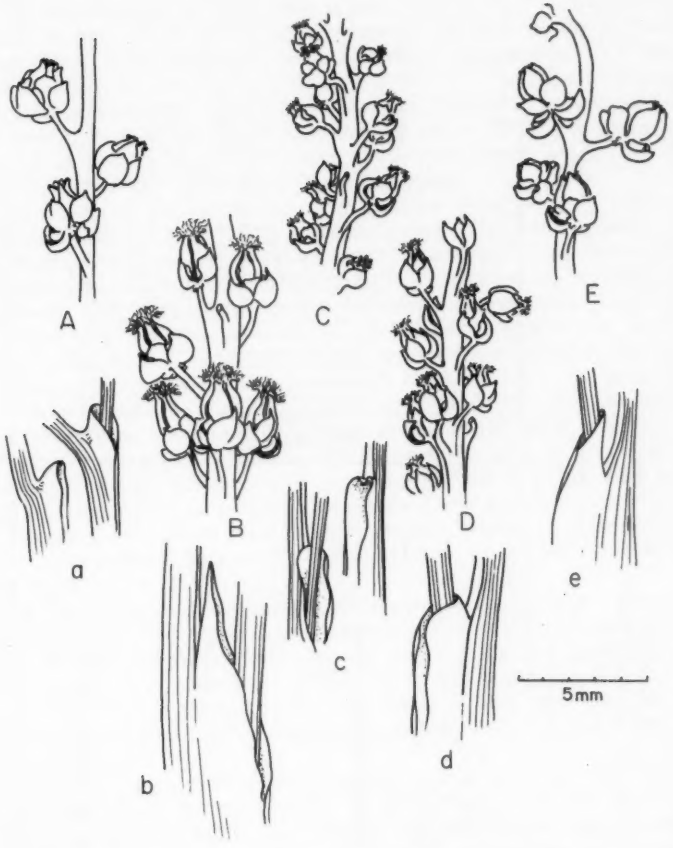

FIG. 2. Flowers and ligules of *Triglochin:* A, *a*, *T. gaspense;* B, *b*, *T. elatum;* C, *c*, *T. concinnum;* D, *d*, *T. debile;* E, *e*, *T. maritimum.* The flowers of *T. maritimum* are further advanced towards fruiting than those of the other species.

bilobed. In *T. debile* (Jones) Löve & Löve the ligule (Fig. 2*d*) is slightly longer than in our small form, often 2 mm long, hood-shaped or square, but in *T. elatum* it is always very long, 4–5 mm, and acute (Fig. 2*b*).

Scapes

The scape of the small plants is slender, $\frac{1}{2}$–1(–1–$\frac{1}{2}$) mm thick, about one-half to one-third of it forming part of the raceme. It is normally shorter than the leaves (Fig. 1). In the tall American and Eurasiatic *Triglochin* the scapes often seem fasciated, but never so in the small plants.

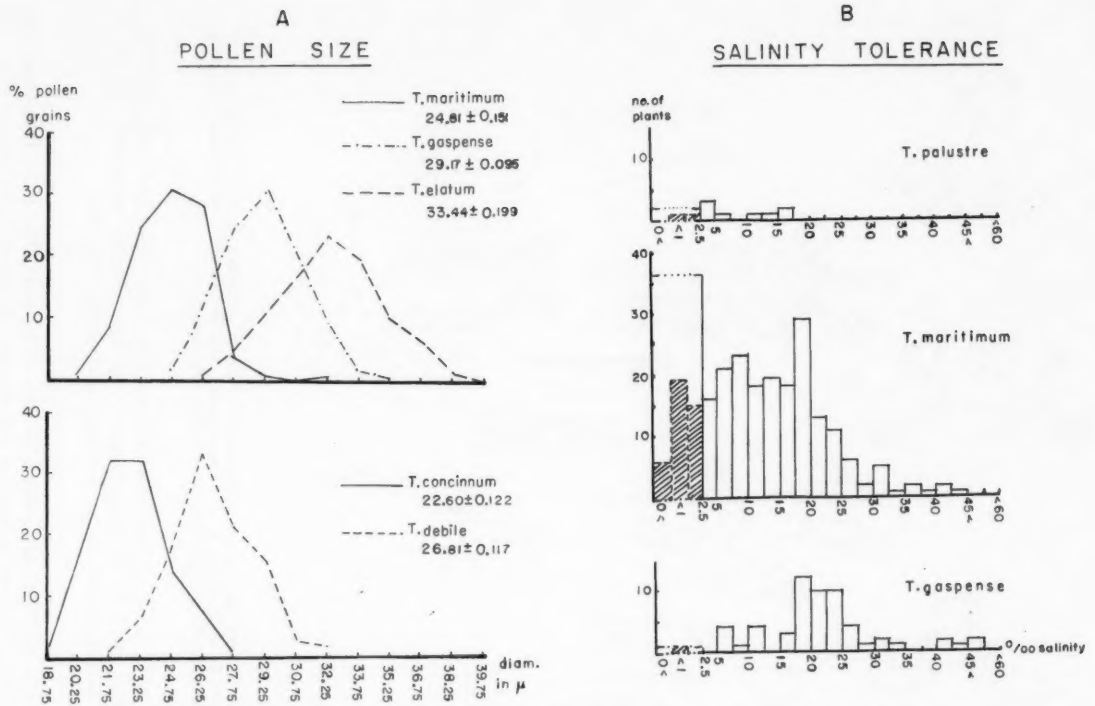

FIG. 3. A. Graphs showing difference in pollen size (largest diameter) between various species of *Triglochin*.
B. Graphs showing differences in salinity tolerance in some *Triglochin* species from Gaspé Peninsula. The first class, 0–2.5, has been split into three parts, marked by hatching of the columns, in order to demonstrate the gradual disappearance of the species towards neutral soil.

Flowers

The inflorescence of *Triglochin* is not yet completely understood (Mason 1957). It has a curious sort of arrangement as each of the six perianthlike appendages, in two series of three, subtends a single stamen. Thus they are not a perianth in the true sense of this word. Whether they are to be regarded as "sepaloid connectives" as in *Potamogeton* (cf. Mason) or as bracts subtending a reduced male flower (Uhl 1947) is beyond the scope of this paper to decide, though it is still a highly interesting matter for further study. We shall refer to them below as "outer" and "inner" perigone.

In our small *Triglochin* the outer and the inner perigone look alike, are roundish (about 1.5×1.5 mm) and cup-shaped. The midribs protrude in a very short apiculus (Fig. 2A). In *T. elatum*, the perigone is approximately similar. The apiculus, however, is sometimes slightly prolonged and whiplike (Fig. 2B). In both, each perigone subtends a fully fertile stamen while in *T. debile* the stamens behind the inner perigone are frequently sterile and shrivelled. All perigone leaves in *T. debile* are oval rather than round and their tips are acutely acuminate (Fig. 2D). This is more easily observed when the inner perigone is flattened against the carpels and not distended by a stamen filled with pollen.

The perigone and stamens are soon caducous and leave prominent scars at the top of the pedicels. To the naked eye these scars look almost like dried remnants of floral parts. Viewed under a magnifier, however, they appear as a complicated system of grooves and ridges. There is no particular difference in the scars of one species from those of another, though in the small plant type they seem perhaps somewhat larger in relation to the pedicel than they do in the other species.

The bilocular stamens dehisce longitudinally and produce liberal amounts of pollen. The pollen grains are round or round-oval with a reticulate surface and no easily visible pores. Sometimes large numbers of oildrops (staining red with Sudan III) are enclosed in apparently fertile grains.

The fertility of the pollen is in most plants very high, usually over 90%. Grains staining red with acetocarmine and well filled with plasma were considered fertile.

Impaired pollen fertility was observed only in plants attacked by fungi, e.g. *Alternaria* sp. (courtesy Drs. R. A. Shoemaker, S. J. Hughes, and L. K. Weresub, Department of Agriculture, Ottawa) or *Puccinia aristidae* Tracy. The latter, a rust, has only been observed by us in plants of *T. debile* from the Prairie Provinces. It causes not only reduced fertility but also stunted growth in the plants affected (cf. Bisby *et al.* 1938). *Alternaria* sp. seems fairly common in occurrence and has been observed in all of the species investigated.

By measuring the size of the pollen in all available, fresh as well as preserved, material of *Triglochin*, we have found a good correlation between chromosome number and pollen size. If other morphological characters fail to give a conclusive indication of whether, for example, a plant is *T. debile* or *T. elatum*, or *T. elatum* or *T. maritimum*, the pollen diameter can be used as evidence. Figure 3A gives graphs showing the variation in pollen diameter in the different species of the *T. maritimum* complex investigated.

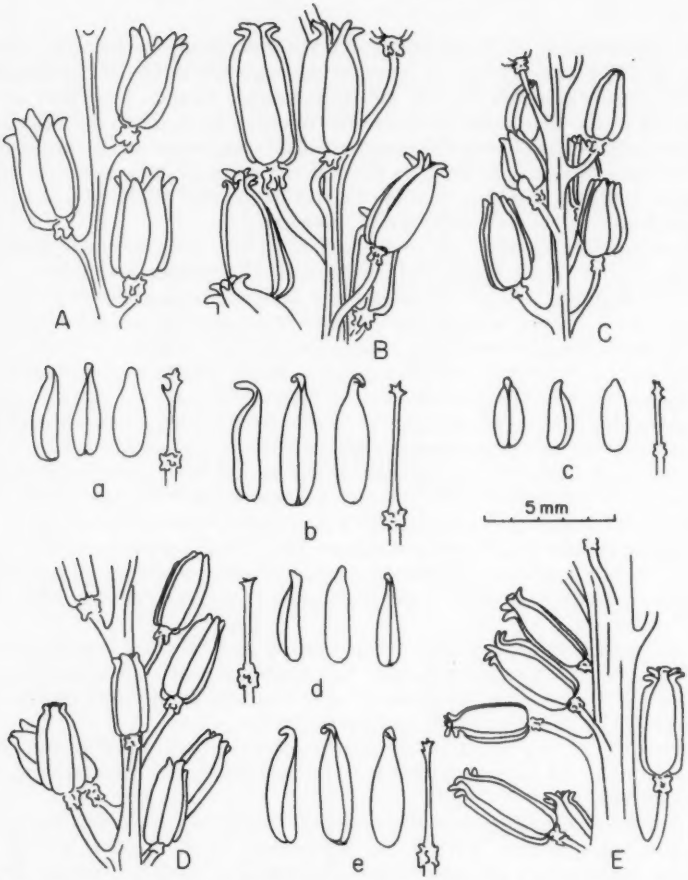

FIG. 4. Fruits and seeds of *Triglochin:* A, *a*, *T. gaspense;* B, *b*, *T. elatum;* C, *c*, *T. maritimum;* D, *d*, *T. concinnum;* E, *e*, *T. debile.*

The carpels of the *Triglochin* sect. *Maritimi* are six in number but from three to twelve have been observed, at least in the dwarf taxon (Fernald 1903; the present authors). In the tall species, reduplications above six have not been noticed by us, though often only three carpels mature to ripe fruits, alternating with three shrivelled and empty carpels. This appears to be particularly frequent in Eurasiatic *T. maritimum* L. (Flora USSR I, 1934). Fewer than six carpels have been observed by these authors in all species of the American part of the *maritimum* complex. This apparent reduction seems to be the result of an uneven fertilization and the consequent abortion of a seed rather than an actual carpel deficiency (Fig. 5*f*).

Even though, in some cases, only three carpels (or fruits) develop, the dwarf plants can neither be referred to *T. palustris* L., nor to the tricarpellate, small and slender-grown *T. striatum* complex of the eastern and southern United States. *T. striatum* has roundish fruits instead of the distinctly three-angled and longish fruits of the *maritimum* complex, and the fruits of *T. palustre* have yet a completely different arrangement and appearance.

In the specimens of our dwarf *Triglochin*, we have also observed a reduplication in the number of carpels, some flowers having as many as 9 to 12. In the cases studied, all flowers of the same raceme have a higher than normal number of carpels, though the number does not seem to be quite constant. It appears, however, that the reduplicated carpels are sterile. We have not been able to verify whether this abnormality is caused by some external agent, but there is no indication, for example, of any fungus present in the epidermal parts of the flowers or in the stalks of the afflicted specimens. No stamens were observed in such abnormal flowers, and the perigone leaves were also abnormal in shape (Fig. 5*e*).

In normal flowers with six carpels, there is a slight difference in stigma type between the various species of the *maritimum* complex. In *T. maritimum* L., the stigma is very small and carries short, glandular hairs, situated on the inner side of a very short, abruptly contracted stigma neck (Figs. 2E; 4 C, *c*). The carpels are plump and the mature fruit measures 2–3(–4) mm long by 1 mm broad. The fruit-bearing axis is short, 2–3 mm, and persistent (Fig. 4*c*).

In *T. elatum* and *T. debile*, the stigma is much larger and the stigma neck longer and more slender, sharply bent outwards so that the large stigma area itself is almost horizontal. The stigmatic, glandular hairs are much longer and, especially in *T. elatum*, copiously bushy (Figs. 2 B, D; 4 B, *b*, E, *e*).

The individual fruits of *T. elatum* and *T. debile* are 4–5(–6) mm or more long, but only 1 mm broad. They therefore appear slender in comparison with the plump fruits of *T. maritimum* L. The persistent central axis is also long, 5–6(–7) mm (Fig. 4 *b*, *e*). *T. concinnum* is very similar to *T. debile* in flower and fruit characters (Figs. 2C; 4 D, *d*, E, *e*) though slightly smaller in all dimensions and with a less bushy stigma (cf. also Fig. 40 in Mason (1957)).

The dwarf Atlantic taxon exhibits somewhat intermediate characters in flowers and fruits (Figs. 2A; 4 A, *a*). The stigma neck is longer than in *T. maritimum* L. and narrows more gradually from the body of the carpel. It bends outwards, but only about 45° and rarely as sharply as in *T. elatum* or *T. debile*. The stigmatic glands are somewhat prolonged and hairlike, but never really bushy-hairy. When mature, the fruits sit less closely together than in *T. elatum* and *T. debile*, and their size is approximately 3–4 mm long by 1 mm broad. The central axis is 3–4 mm tall. Sometimes the embryo does not completely fill the fruit, causing it to appear slightly winged.

In our greenhouse, we have observed that without artificial pollination the small plants do not set seed. When pollinated by hand, however, they produce mature fruits even with their own pollen.

Cytology

There is a certain superficial resemblance between small specimens of *T. concinnum* from California and the dwarf taxon from the American east coast,

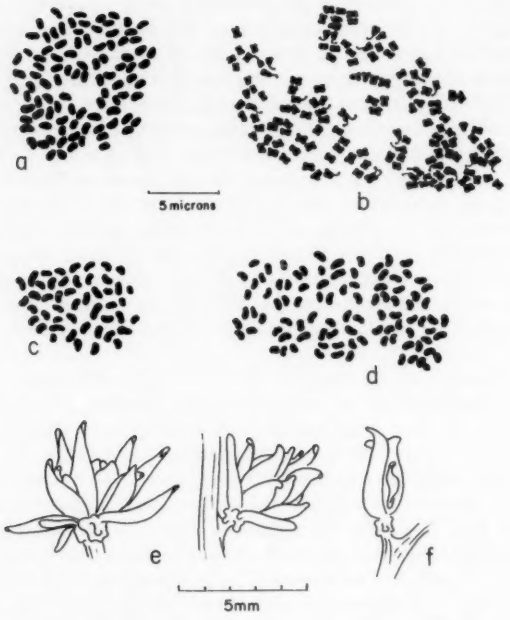

FIG. 5. *a–d.* Chromosomes of *Triglochin: a, T. gaspense*, $2n = 96$; *b, T. elatum*, $2n = 144$; *c, T. concinnum*, $2n = 48$; *d, T. debile*, $2n = 96$. (*a, c, d*, cut sections, stained with Gentian violet; *b*, squash preparation, stained with aceto-orcein.)
e, f. Abnormal carpels of *T. gaspense: e*, reduplication; *f*, reduction in number of carpels.

as may be seen when comparing our Fig. 1 and, for example, Fig. 40*d* in "A flora of the marshes of California" (Mason 1957). But closer scrutiny reveals several distinct differences between the two. Any close relationship is, furthermore, ruled out by the fact that the Californian *T. concinnum* has $2n = 48$ chromosomes (Löve and Löve 1958) while the new taxon has $2n = 96$ (Fig. 5 *a, c*). *T. concinnum* Davy is a good species (Howell 1947; Löve and Löve 1958) and should not be confused with the Atlantic taxon (Fernald 1950).

Neither is it feasible to consider the small taxon as a depauperate ecological form (Fernald 1903, 1950) of the common *Triglochin* of the Atlantic east coast, *T. elatum*, as this species has $2n = 144$ chromosomes (Fig. 5*b*; cf. also Löve and Löve (1958)). *T. debile* from the interior of the continent and the Pacific coast, however, has $2n = 96$ chromosomes (Fig. 5*d*; cf. also Löve and Löve (1958)), but can only be regarded as remotely related to the small Atlantic taxon with the same chromosome number. Instead, *T. debile* may be considered as a polyploid of the Pacific *T. concinnum*.

Ecology

As already noted by Fernald (1903), the dwarf taxon grows in salt marshes, normally within the tidal zone and below the high-water mark. In most cases, where we have observed it, it is completely submerged twice daily. The taxon is most abundant around small lagoons inside the salt marshes in a certain ecological belt, characterized by a dwarf meadow of, usually, succulent plants. The dominant species of this community are *Glaux maritima* L., *Plantago oliganthos* R. & S., and the new *Triglochin*. A more detailed account of the habitat will be given later in a description of all the Gaspé Peninsula salt-marsh communities (Lieth and Dansereau, unpubl.).

Even while studying the correlation between salt-marsh plants and certain degrees of salinity, the problem arose whether to regard the new taxon as a species or as a race only. The result of the salinity tolerance investigations (Lieth, unpubl.) shows clearly that the two taxa of the *T. maritimum* complex present in these marshes occupy different ecological amplitudes within the available salinity gradient (Fig. 3B). The ranges of both these taxa differ also from that of *T. palustre* (same figure).

It is true that there is a good deal of overlap in the tolerance of salinity where the two taxa of the *maritimum* complex are found, but they exhibit a distinctly different preference (Fig. 3B).

Fernald (1903) mentioned, and we, too, have observed that because of the greater salinity and the correlated higher osmotic value of the soil/water solution, the normally tall *T. elatum* around the inner edges of the salt marsh becomes smaller and smaller as it penetrates further seawards into the marsh. However, one important morphological feature remains unchanged: *T. elatum* always forms hummocks, whereas the small taxon grows as a "dwarf lawn". When under extreme conditions the spikes of *T. elatum* become very depauperate, it is not always easy to distinguish between herbarium specimens of the two taxa from the same type of habitat. Normally, the spike of even depauperate *T. elatum* is denser than that of the small taxon, but the difference in pollen size (Fig. 3A) remains constant under all circumstances and can be measured also from dried specimens.

Thus the ecological specialization of the dwarf *Triglochin* corroborates the morphological and cytological indications that we are dealing with a new species.

Distribution

Geographically, it seems that the area of the new taxon is limited to Quebec and the Canadian Maritime Provinces, Newfoundland, and the state of Maine, U.S.A. (Fig. 6). Most likely it may be considerably more common in nature than its occurrence in herbaria indicates at present. Its habitat may be the inhibiting factor which has limited the number of collections. Not too many botanists venture out into mucky salt marshes, and few would pick up what at first glance appears like some reduced and "common" *Triglochin*. Besides, as we have concentrated our studies on material in Canadian herbaria only, the possibility that more localities may be found along the New England coast is not excluded. The southern limit of the new taxon must, therefore, be regarded

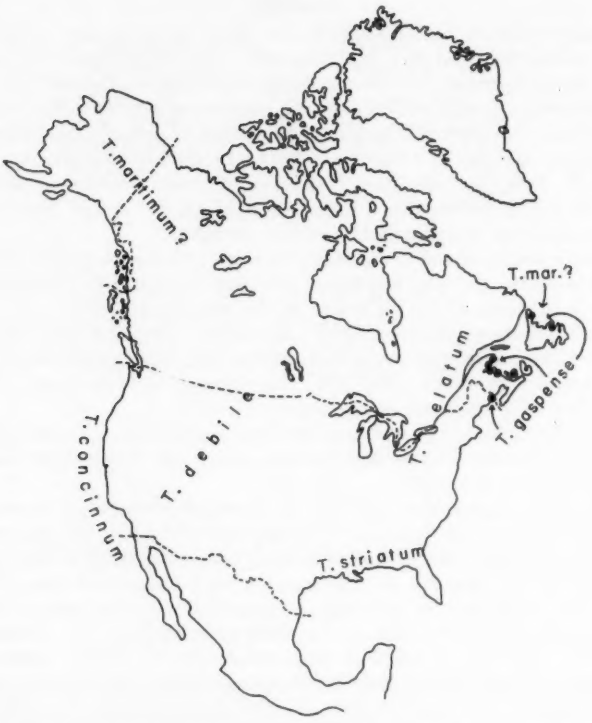

FIG. 6. Map showing the approximate distribution in North America of the species of the *Triglochin maritimum* complex which have been mentioned in the text. The small dots indicate the presently known localities of *T. gaspense*.

as not yet fully established. We assume, however, that Newfoundland represents the northern limit of its area, as we have seen no sample from either the north shore of the Gulf of St. Lawrence or Labrador.

The following specimens are deemed to be representative of the new taxon (MT = Marie-Victorin Herbarium, Université de Montréal; DAO = Herbarium of the Department of Agriculture, Ottawa; no specimens were located in the Herbarium of the National Museum of Canada, Ottawa, but one of our specimens has been deposited there):

Newfoundland

1. Beaver Brook, Canada Bay. "High tide level in gravel beach. Some large patches of very small plants, these only flowering ones evident". July 17, 1956. No. 30, Donly (DAO).

2. Salt-pond near Traytown, Bonavista North distr. "Edge of tidal pond". July 23, 1945. No. 297, Smith, Smith, Squires (DAO).

3. Wolfe Island, Alexander Bay, Bonavista North distr. "Sandy beach". July 21, 1945. No. 253, Smith, Squires (MT).

Quebec

4. Between Barachois-Ouest and Coin-du-Banc, Gaspé Peninsula. "Salt marsh below high-water mark". July 25, 1960. No. 60–0636, Lieth, Dansereau, Waltz, Ouelette (Type, MT).

D:o, July 11, 1960. Nos. 60–0630 and 60–0381, Dansereau, Waltz, Ouelette (MT).

D:o, July 25, 1960. Nos. 60–0645, 60–0648, 60–0651, Lieth, Dansereau, Waltz, Ouelette (Paratype, MT).

5. Malbaie, Gaspé Peninsula. In tidal zone below high-water mark. Aug. 18, 1959, Dahl (MT).

6. St. Omer, Gaspé Peninsula. In tidal zone below high-water mark. Aug. 18, 1959, Dahl (MT).

New Brunswick

7. Near Caraquet, Gloucester Co. "Saline marsh among *Spartina alterniflora*. (Leaves and stems upright; leaves terete, soft and spongy)". July 20, 1945. No. 45.651, Dore, Gorham (MT, DAO).

8. Tête-à-Gouche River, Gloucester Co. "Estuary marsh". 1902. Fernald, Williams (*in litt.*, cf. Fernald 1903).

Prince Edward Island

9. Souris, King's Co. "(Of the *T. concinna*-type.) Mudflats of salt marsh". Aug. 4, 1952. No. 1467, Erskine (MT, DAO).

Maine

10. Schooner Cove, Cutler, Maine. July 1902. Fernald, Kennedy, Collins, Williams (*in litt.*, cf. Fernald 1903).

Conclusions

From what has been written above it is evident that the small Atlantic *Triglochin* (Fig. 1) distinguishes itself morphologically, ecologically, and cytologically from all other North American species of the *T. maritimum* complex, and that it has a distinct geographical area. Therefore, we propose the name *Triglochin gaspense* for this entity, as it so far has been most frequently collected and studied in the Gaspé Peninsula, a locality which seems to be centrally located within its distribution area (Fig. 6).

Triglochin gaspense Lieth & D. Löve n. sp.

Differt ab aliis speciebus affinibus gregis *T. maritimi* statura minima, matura (5–)10–15(–20) cm alta; folia scapum aequantes vel superantes, e vaginis 30–45° recurvatis; spica breva, (2–)3–5(–7) cm longa, sed laxiflora, (3–)5–12(–30) flores gerens. Numerus chromosomorum 96. Diameter granorum pollinis 29 μ. Habitat in regione inundata inter fluxos maris in Provinciis canadensibus maritimis atlanticis nec non in Mainense. Typus in Herbario Mariae-Victorini, Montis Regis, Canada.

Acknowledgments

The authors wish to express their gratitude to Dr. Eilif Dahl, Norway, for putting both fresh material and his collection of *Triglochin* from the Gaspé Peninsula at our disposition. Furthermore, we wish to thank our friends and colleagues of the Institut Botanique, Université de Montréal, as well as of the Jardin Botanique de Montréal, who have helped us in various ways during the present study. We also want to thank the National Research Council of Canada for financial assistance which made this work possible.

References

BISBY, G. R. *et al.* 1938. The fungi of Manitoba and Saskatchewan. National Research Council, Ottawa.

FERNALD, M. L. 1903. Some variations in *Triglochin maritima*. Rhodora, **5**, 174–175.

——— 1950. Gray's Manual of botany. 8th ed. American Book Company, New York.

FLORA USSR I. 1934. *Triglochin*. Moscow–Leningrad. pp. 276–277.

HOWELL, J. T. 1947. Remarks on *Triglochin concinna*. Leafl. of Western Botany, V, 13–19.

LÖVE, A. and LÖVE, D. 1958. Biosystematics of *Triglochin maritimum* agg. Naturaliste can. **85**, 156–165.

MASON, H. L. 1957. A flora of the marshes of California. University of California Press.

UHL, N. W. 1947. Studies in the floral morphology and anatomy of certain members of the Helobiae. Dissertation, Cornell University.

TEMPERATURE AND SEXUALITY IN CHLAMYDOMONAS CHLAMYDOGAMA[1]

FRANCIS R. TRAINOR

Abstract

The effect of various temperatures on the mating reaction in *Chlamydomonas chlamydogama* was determined. When mixed mating types were suspended in an ammonium nitrate deficient medium at 30° or 34° C for 30 hours of continuous illumination and then shifted to 22° C, an optimal mating reaction could be obtained. Although high zygospore percentages could be obtained with other temperature combinations, a temperature drop was necessary under continuous illumination. The mating reaction, from clump formation to zygote production, was shown to take place at the lower temperature.

Although for many years *Chlamydomonas* species have been used for mating experiments and in genetical studies, little is known of the effect of temperature on various stages in the mating process. Previous investigators (3, 4, 5, 6) have used temperatures of between 12° and 26° C for investigations with *Chlamydomonas* but a constant temperature was always used for mating experiments.

With *Protosiphon botryoides*, Klebs (5) indicated that with increased temperature less time was needed for gamete formation. With the same species, Maher (7) found that the number of hours required for gamete formation decreased from 8 to 4 with a temperature change of 18.5° to 28° C for 1 hour. She also stated that an increase in temperature of 5–10° C above that at which the plants had been grown always brought about gamete formation in plants more than 2 weeks old.

During mating experiments with *C. chlamydogama* (11) it has been demonstrated that a temperature as high as 34° C can be used for a number of hours, provided the mixed mating types were subsequently stored in darkness at 22° C for a number of hours. Good zygospore yields were obtained when either 30° or 34° C was used for at least 30 hours, while progressively smaller yields resulted when lower temperatures were tested for that same time-period. Microscopic observations indicated that the mating reaction was not initiated until the mixed mating types were stored in darkness at 22° C. At that time it was not clear whether storage in the dark or the temperature of 22° C, or both, were responsible for the mating reaction.

In the present paper, the results of experiments with the dark period at 22° C are reported. In addition, the effect of temperature on growth of the mating types, the effect of various temperatures during the 30-hour period, and the effect of temperature during the time mating itself occurs (after the 30-hour period) are reported.

Materials and Methods

The mating types of *C. chlamydogama* were clonal cultures of the Bold strains (1) W16–1 and W16–2 maintained by the writer since 1954. Growth

[1]Manuscript received April 28, 1961.
Contribution from the Department of Botany, University of Connecticut, Storrs, Conn.

and harvesting media and the harvesting procedure have been described previously (10).

Only W16–1 was used for the experiments on growth. All of the growth studies were conducted at an intensity of 600 ft-c of fluorescent illumination in constant-temperature cabinets. The fluctuation in temperature did not exceed $\pm 0.5°$ C in these chambers. Cells were suspended in 10 ml of growth medium in 125-ml flasks. During growth these flasks were continuously agitated at a constant speed on a wrist-action shaker. The arm of the shaker entered the constant-temperature cabinet through a small opening in the wall. Cells were dispersed in a Spencer counting chamber for cell counts at regular intervals.

For all other experiments with mating, cultures also were illuminated with both 40 w G.E. "Standard Cool White" and "Daylight" fluorescent lamps, but at an intensity of 400 ft-c, at which optimal mating reactions can take place (10).

Mating experiments were conducted in the constant-temperature cabinets with temperature control of $\pm 0.5°$ C.

When the zygospores were formed in a pellicle, the contents of each petri dish were placed in a tissue grinder (A. H. Thomas Co.). A half dozen strokes with the teflon bar destroyed the pellicle. When the mixture was then dispersed in a Spencer counting chamber, accurate counts of zygospores and vegetative cells could be made. Calculations were then made of the percentage of total possible zygospores present (10).

Figures reported in the graphs and tables are averages of from two to four separate experiments.

Results

Previous experiments with *Chlamydomonas chlamydogama* have been reported in which the mixed mating types were placed in petri dishes at temperatures of from 22° to 38° C. Samples were taken out of the light at regular intervals and stored in darkness at 22° C. After 24 hours they were fixed and the percentage of zygospores was calculated. In the first experiment reported here, dishes of mixed mating types were placed in the light at 34° C only, and at regular intervals mixed mating types were taken out of this temperature and placed in both the light and the dark at 22° C. The results of this experiment are shown in Fig. 1. These results, along with those previously reported (11), indicate that the temperature drop initiated the mating reaction. Mating can occur in darkness, but at all points higher yields resulted when illumination was constant during the temperature drop.

As shown in Fig. 2 mixed mating types were placed in three different temperatures for varying periods of time and then placed in the light at 22° C for 24 hours before calculations of zygospore percentages were made. These curves follow the same pattern as those previously reported (11) when the 24-hour period at 22° C was in darkness. However, at all points, higher zygospore percentages are recorded, and all demonstrate that darkness is not necessary for mating.

For convenience during the following experiments, the higher temperature used (e.g., 34° C) is called the "starvation" temperature and the lower (22° C) the "mating" temperature.

With the use of the shift in temperature under continuous illumination, one disadvantage accrued. Trainor (11) reported that the mating reaction was not initiated until the dishes were stored at 22° C. When the dishes at 22° C were illuminated, as under the present conditions, zygospores were always formed in a rather dense pellicle. At first this made calculations impossible.

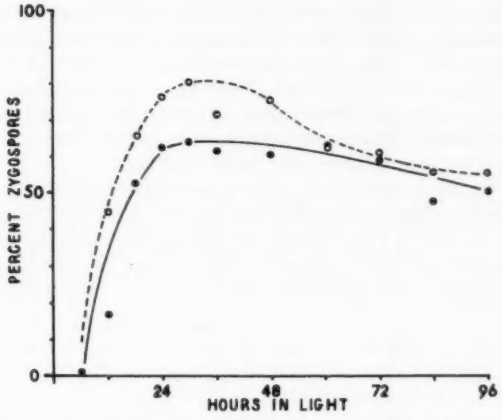

FIG. 1. Mating in *Chlamydomonas chlamydogama*, in which cells were taken out of the 34° C temperature at the points indicated on the graph and placed at 22° C in the light (upper curve) and at 22° C in the dark (lower curve) for 24 hours.

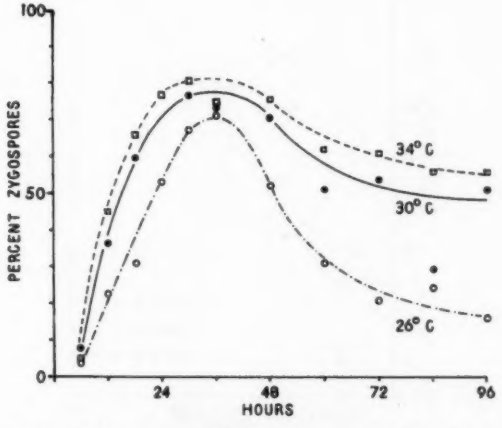

FIG. 2. Mating in *Chlamydomonas chlamydogama* in which cells were periodically taken out of the three different temperatures listed and placed in the light at 22° C for 24 hours.

A series of experiments were conducted in an attempt to eliminate, or break up, this aggregation. A wide range of concentrations of both Duponol (DuPont) and Tween 80 (Atlas Powder Co.) were used and were unsuccessful. Satisfactory results were obtained when the pellicle and vegetative cells were placed in a tissue grinder. After a few manual strokes, cells could be dispersed in a counting chamber and accurate zygospore percentages calculated.

At this point experiments were conducted with temperature and growth of the W16–1 mating type. These investigations were not designed to obtain maximal growth rates, but rather to observe the effect of temperature on growth under standard conditions. Once the temperatures at which good growth could be obtained were known, it could be determined whether the temperature at which the mating types were grown had any effect on the results obtained during "starvation" and "mating".

The points for the growth rates at various temperatures (Fig. 3) are plotted as $\log_2$ of the cell number over a 60-hour period. Two doublings per day were obtained at 22° C and 2.5 doublings per day at both 26° and 30° C. At 34° C little or no growth was noted. These results are not plotted inasmuch as some cells were bleached, and probably dead, and, in addition, daughter cells

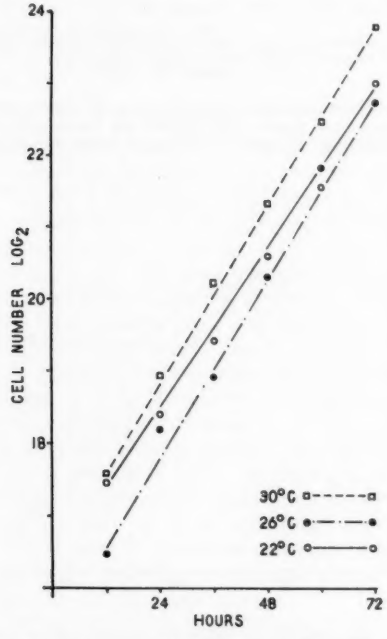

FIG. 3. Growth of *Chlamydomonas chlamydogama* at three different temperatures; cell number is plotted as $\log_2$.

TABLE I

Percentages of zygotes of *Chlamydomonas chlamydogama* resulting when different growth, "starvation", and "mating" temperatures were used

Growth temp., ° C	"Starvation" temp., ° C	With "mating" temp. of:			
		18° C	22° C	26° C	30° C
22	26	44%	45%	18%	12%
	30	59%	66%	55%	13%
	34	68%	85%	73%	17%
26	26	34%	43%	23%	12%
	30	66%	85%	67%	13%
	34	78%	81%	74%	49%
30	26	32%	43%	23%	13%
	30	62%	77%	60%	18%
	34	78%	77%	68%	31%

usually were not released from the mother cell at this temperature. With these large sporangia accurate cell counts were not possible.

Next a series of cultures were grown at 22°, 26°, and 30° C and subjected to various "starvation" temperatures, namely, 26°, 30°, and 34° C. After 30 hours, cultures were placed at different "mating" temperatures for 24 hours. The results are summarized in Table I. It appears that a change in temperature of growth had little or no effect on results obtained later, during the temperature shift from the "starvation" to the "mating" temperature. In addition, the best "mating" temperature seems to be at least as low as 22° C, with some high yields also apparent at the 18° C mating temperature.

In Fig. 4 the information is plotted for zygospore yields of cultures grown at 26° C for 3 days and subjected to three "starvation" temperatures and four

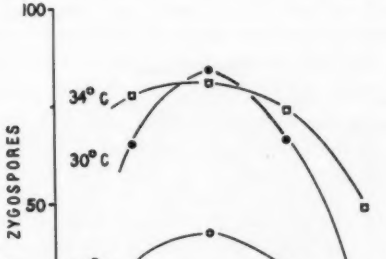

FIG. 4. Mating in *Chlamydomonas chlamydogama* in which cells were grown at 26° C; three "starvation" temperatures were used (26°, 30°, and 34° C) for 30 hours; and 18°, 22°, 26°, and 30° C "mating" temperatures were used.

"mating" temperatures. This graph seems to indicate that even though the best zygospore yields are obtained at 22° C this temperature is not an absolute requirement for sexuality. It appears that at higher "starvation" temperatures a wider range of "mating" temperatures will give a good zygospore yield.

Now an attempt was made to determine at what point clumping occurred and zygotes were first present when the mixed mating types were at the "mating" temperature. In addition, a comparison was made of the performances at two "mating" temperatures, 18° and 22° C.

Cultures were grown at 26° C and a series of petri dishes were exposed to a 34° C "starvation" temperature for 32 hours. Half of the dishes were placed at a "mating" temperature of 18° C and half at 22° C. Periodically, observations were made and zygote percentages calculated. These results are presented in Table II.

From these data it is clear that the sexual reaction is slowed down at 18° C, and that at 22° C most of the zygotes are formed within a 3-hour period.

TABLE II

A comparison of clumping and zygote production in *Chlamydomonas chlamydogama* using two different "mating" temperatures. Prior to this the mating types were grown at 26° C, mixed, and placed at 34° C for 32 hours

	With mating temp. of:	
	18° C	22° C
Small clumps (5–10 cells)	4 hr	3 hr
Good clumps (over 50 cells)	5 hr	4 hr
First zygotes	9 hr	7 hr
50% zygotes	12 hr	9 hr
Maximal yield	ca. 13 hr	10 hr

Discussion

The data presented in Fig. 1 indicate that the shift in temperature from 34° to 22° C stimulates the mating reaction, which occurs at the lower temperature. Inasmuch as the percentages recorded in the lower curve are above 60% for at least a 36-hour period, it is clear that light is not necessary for the completion of the reaction. However, in the presence of light (upper curve), the zygospore percentages are always higher. Perhaps this increase in yield, produced by the additional light at the lower temperature, can be explained by postulating an extra division of some cells at the lower temperature in the presence of light, and no division when a dark period is substituted. The products of these divisions could act as gametes. This could be checked by cell counts.

Up to the point where the "starvation" temperature becomes lethal, higher temperatures always are responsible for higher zygospore yields. The reactions start off more rapidly, reach higher peaks, and taper off more slowly when the higher "starvation" temperatures are used. Yet at the 30–36-hour period at least a 70% reaction can be recorded with any of the three temperatures used here.

Once it had been determined that the shift in temperature initiated the mating reaction, it became apparent that temperature of growth must be investigated. It could well be that the preconditioning of cells, prior to the temperature shift during "starvation" and "mating", would have some effect on what optima could be expected. The growth rate at 26° and 30° C was slightly better than at 22° C and was approximately 2.5 doublings/day.

When an inoculum of about 100,000 cells/ml was used at 34° C, little growth was recorded; some cells were bleached and others in obviously poor condition. A higher initial inoculum was then used because of the favorable condition of cells in previously described mating experiments in the nitrate-deficient medium at the same temperature. Under those conditions about 6 million cells/ml were used. With an inoculum of 1 million cells/ml a growth rate of approximately 1 doubling/day was obtained. Perhaps, with the smaller inoculum, the light intensity was too strong at the near-lethal temperature. Inasmuch as the daughter cells at 34° C would tend to be enclosed in the mother cell wall, counting was difficult and the results only approximations. However, the growth rate never approached that found at the lower temperatures.

The experimentation which followed, that which tested the effect of temperature of growth on the later temperature shift, made it apparent that growth temperature had little effect on what followed. That is, there was no preconditioning of cells during the 3 days of growth at the three different temperatures. It also seems clear from the same results in Table II that the higher "starvation" temperatures, when combined with the lower "mating" temperatures, result in the best zygospore yields.

In view of the fact that growth was rather poor in the complete medium at 34° C, one wonders what effect 30 or more hours at 34° C in a nitrate-deficient medium, the harvesting medium, has on the formation of gametes. It appears at present that at least some cells after division do not release daughter cells at the "starvation" temperature, but only do so when placed at the lower "mating" temperature. These newly released daughter cells in the favorable medium can act as gametes.

Because temperature shifts have been used to effect synchronization in some organisms (2, 8, 9), cell counts were made periodically to determine whether there was some sudden increase in cell number during this temperature shift. Preliminary investigations indicate that this is not the case. However, some modification of the temperature, illumination, or time periods might induce some synchrony. It appears at present that release of daughter cells is gradual, and that other factors, e.g. stimulation of motility and possible release of a clumping substance, also play an important part in the eventual production of a high percentage of zygospores.

The data presented in Table II seem to indicate that once the mating reaction begins, it proceeds rather rapidly. A few pairs were noted as early as 5 hours at 22° C, but it was not until 7 hours that the count was more than a few per cent; at that time 40% pairing was noted. Inasmuch as pairing is not of long duration in this species and the maximal reaction was noted 3 hours later, the zygotes which result are all about the same age.

At first it seems difficult to understand why such a drastic shift in temperature results in an excellent zygospore yield. This is true especially if one recalls the earlier report (10) that diurnal illumination stimulated an excellent yield in this same species. A subtle temperature shift must have been present in the laboratory when diurnal illumination was used. In that former laboratory constant-temperature cabinets were not available. With the sensing bulb of a continuous recorder directly under the lamps which are used for experimentation, one can demonstrate diurnal changes in temperature, especially if the diurnal illumination used is "on" during the day.

This temperature shift under diurnal illumination, although not as drastic as that reported here, but *repeated daily*, possibly would have the same effect as the 34° to 22° C temperature shift. It is also possible that the mating reaction in this species can be initiated by various combinations of temperature and illumination.

A good sexual reaction can be obtained with *Chlamydomonas chlamydogama* with growth of mating types at 26° C for 3 days, harvesting of cells into a nitrate-deficient medium, storage at 34° C for 30 hours, and finally a temperature shift to 22° C for 12 hours. A yield of approximately 80% zygospores will result.

References

1. Bold, H. C. 1949. The morphology of *Chlamydomonas chlamydogama*, sp. nov. Bull. Torrey Botan. Club, **76**, 101–108.
2. Campbell, A. 1957. Synchronization of cell division. Bacteriol. Revs. **21**, 263–272.
3. Ebersold, W. T. 1956. Crossing over in *Chlamydomonas reinhardi*. Am. J. Botany, **43**, 408–410.
4. Förster, H. and Wiese, L. 1954. Gamonwirkung bei *Chlamydomonas eugametos*. Z. Naturforsch. **9b**, 548–550.
5. Klebs, G. 1896. Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Fischer, Jena.
6. Lewin, R. A. 1956. Control of sexual activity in *Chlamydomonas* by light. J. Gen. Microbiol. **15**, 170–185.
7. Maher, Sister M. L. 1947. The role of certain environmental factors in growth and reproduction of *Protosiphon botryoides Klebs*. III. Reproduction. Bull. Torrey Botan. Club, **74**, 156–179.
8. Scherbaum, O. and Zeuthen, E. 1954. Induction of synchronous cell division in mass cultures of *Tetrahymena piriformis*. Exptl. Cell Research, **6**, 221–227.
9. Scherbaum, O. H. 1960. Synchronous division of microorganisms. Ann. Rev. Microbiol. **14**, 283–310.
10. Trainor, F. R. 1958. Control of sexuality in *Chlamydomonas chlamydogama*. Am. J. Botany, **45**, 621–626.
11. Trainor, F. R. 1960. Mating in *Chlamydomonas chlamydogama* at various temperatures under continuous illumination. Am. J. Botany, **47**, 482–484.

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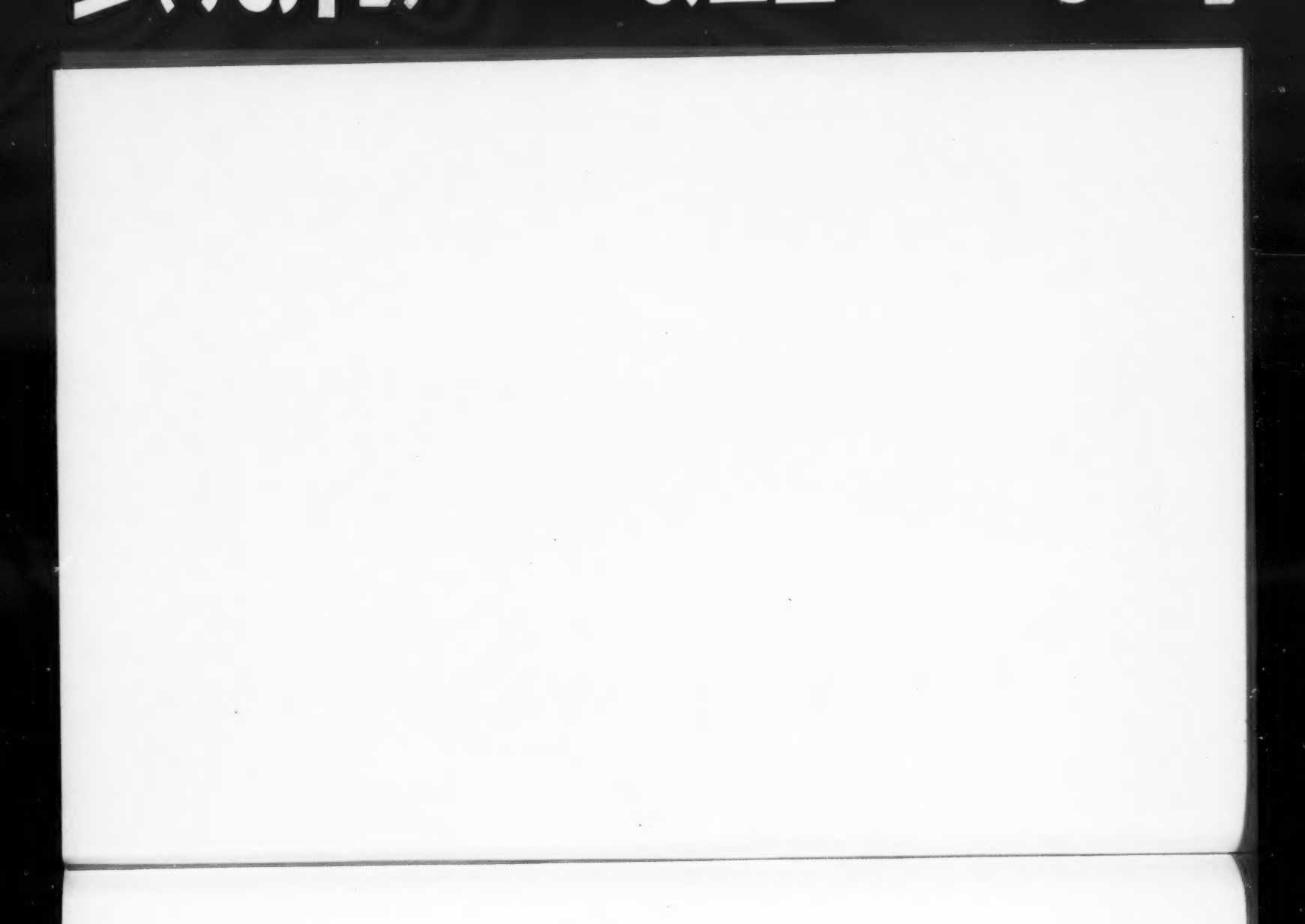

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